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3976

Influence of Oral Administration of Bile Upon Fasting Gastric Acidity and Intestinal Bactericidal Action.

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Living non-pathogenic bacteria appear in the thoracic duct lymph when these microorganisms are placed in the lumen of the duodenum in an alkalinized bile solution in the post-digestive dog.[1, 2] Various concentrations of bile have been placed in the stomach of dogs through a fistulous opening and the gastric contents removed and titrated at intervals 2 to 3 hours after the bile was administered. Dehydrated bile (Difco) has been used, 100 cc. volume of fluid, concentration of bile varying from 0.5 to 10.0%. It was found that 5.0% and higher concentrations caused vomiting within a short period of time, followed by diarrhea. There was usually a period of active HCl secretion following the emptying of the stomach in this manner. One half of the required amount of acid was added to neutralize the alkali in 100 cc. of a 5.0% bile solution to see if this mixture would leave the stomach rapidly and not stimulate acid secretion. (Line "D" in graph.) One per cent bile was found to be the optimum concentration to rapidly leave the stomach and be followed by a certain period of acid deficit. (Line "B" in graph.) Three dogs were used for these experiments. Each

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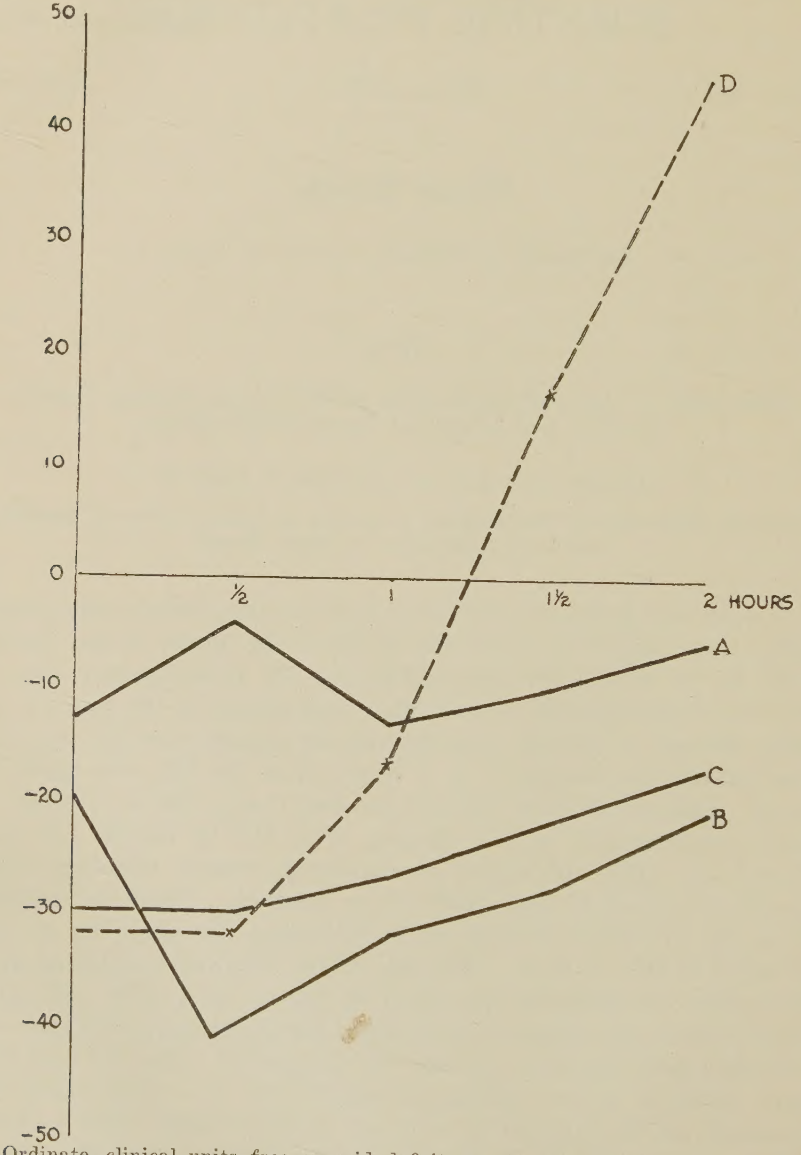
[1] Arnold and Brody, PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 247.

[2] Arnold, *Am. J. Hygiene*, 1928, in press.

curve on the graph is an average of 5 experiments using 3 dogs, a total of 15 separate experiments. Experiments were conducted during the forenoon. Animals were fed in the afternoon and used on alternating dogs 18 hours after feeding.



Ordinate, clinical units free or acid deficit per 100 cc. gastric contents. Abscissa, time in half hour periods. A. Curve, 100 cc. distilled water + 1 agar petri dish of *B. prodigiosus*. B. Curve, 100 cc. 1% bile + same bacteria. C. Curve, 100 cc. 2% bile + same bacteria. D. Curve, 100 cc. 5% bile plus sufficient acid to neutralize half of the alkaline content + same bacteria. The acid-deficit of the mixture placed in the stomach is indicated at the beginning of the curve.

These animals had, in addition to the gastric fistula, a caecal fistula.[2] *B. prodigiosus* was suspended in water and the various bile solutions and the relative concentration in the caecum was determined. This method has been used to determine the relative bacterial killing power of the intestinal tract.[2] These test microorganisms appeared only in 15 to 20% of their original concentration in the caecum for about 2 hours (1½ to 3½ hours after feeding) when given in distilled water. In 1% bile they appear in the caecum within 30 minutes and last for 6 hours and are in the same concentration as was placed in the stomach. There is no evidence of a bacterial killing action in the passage from the stomach to the caecum. This concentration of bile is below the diarrhea dose. The test bacteria can be cultured from the gastric contents so long as there is an acid-deficit in the stomach. Higher concentrations of bile that produce vomiting and diarrhea are associated with an interference with the bactericidal power of the intestinal tract, but the period is shorter in duration. This is probably due to the gastric acid secretion that follows these stimulating doses of larger quantities of bile. One gram of dried bile in 100 cc. of water leaves the post-digestive dog's stomach rapidly and is followed by a period of achlorhydria. This causes a change in the normal reaction of the small intestine by allowing alkaline buffered solution to pass into the duodenum and is associated with an interference with the normal bacterial killing power of the small intestine. This is a preliminary report upon the problem of immunization by oral administration of the vaccine.

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Further Observations on Mammillo-infundibular Region of Diencephalon: Relation to Epilepsy, Dementia and the Psychoses.

LAWRENCE O. MORGAN. (Introduced by V. E. Emmel.)

From the Department of Anatomy, University of Illinois College of Medicine.

In a recent report by Morgan and Johnson[1] it was shown that a lesion in the mammillo-infundibular region of the brain in dogs was followed by symptoms which were in all essential respects identical with epilepsy in the human. The author[2] has reported on a study of

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[1] Morgan, Lawrence O., and Johnson, Clarence A., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 442.

[2] Morgan, Lawrence O., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 444.

4 epileptic brains in which there were marked degenerative changes in the same nuclei which were injured by experimental lesions in the dog *(substantia grisea* of the third ventricle, *nucleus mammillo-infundibularis,* and *nucleus tuberis).*

The epileptic fit as produced experimentally in the dog lasts from 1½ to 3 minutes and is characterized by first tonic and then clonic spasms, unconsciousness, dilation of pupils, salivation, vasoconstriction, increased heart rate, rise in temperature and increased blood pressure. The fits usually commence a few hours after the operation and become more frequent and severe until the animal passes into a condition resembling *status epilepticus.* At this stage the intestines and stomach are dilated, the heart rate increased to 200-250 and the temperature rises to 105-110. In 3 cases clamping off the blood supply to one or sometimes both suprarenals caused an immediate cessation of all symptoms involving the smooth and cardiac musculature and partially inhibited the convulsions which involved the voluntary musculature. Clamping off one or sometimes both thyroids (including parathyroids) stopped the spasms of the voluntary musculature but had no appreciable effect on the remaining symptoms. These animals lived for some time but none of them recovered following the clamping off of the glands.

In one animal which showed the usual symptoms except that the muscular spasms were almost entirely absent the lesion was confined to the *substantia grisea* of the third ventricle with little or no injury to the remaining nuclei. These symptoms disappeared (except the return of consciousness which was only partial) when the left suprarenal was clamped off.

I wish to report an additional case of epilepsy in which the mammillo-infundibular region of the brain shows the same changes previously described.

Human brains have been examined in one case of katatonia dementia precox, one of psychosis with progressive mental deterioration (dementia), and 3 cases of psychosis accompanying cerebral arteriosclerosis. In all of these cases the cells of the *substantia grisea* of the third ventricle are reduced to from 1/3 to 1/2 of the normal number. Many of the remaining cells are chromatolytic and there is some shrinkage and marked proliferation of *glia* in the area occupied by this nucleus. The *nucleus tuberis* shows a varying amount of chromatolysis but no cell destruction.

The data obtained from experimental animals and from a study of pathological human material, although perhaps not sufficient to be conclusive is quite suggestive. The *substantia grisea* of the third ventricle is probably a secretory center for the suprarenal glands and seems to be concerned in some way with the dementias and psychoses and is probably the particular cell group which is concerned with the mental deterioration common to epilepsy. This nucleus is also concerned with the epileptic fit; especially such symptoms as dilation of the pupil, frothing at the mouth, cardiovascular disturbances and perhaps loss of consciousness through a disturbance of the cerebral blood supply. The nucleus *mammillo-infundibularis* and *nucleus tuberis* are probably secretory centers for the thyroid and parathyroid and are concerned with the muscular spasms characteristic of the epileptic fit and perhaps also in body temperature. There is as yet no indication as to which of these nuclei is a center for the thyroid and which for the parathyroid. It is likewise impossible to say what specific part each gland plays in the symptomatology. The epileptic fit is probably the result of a hyperactivity of the 3 nuclei of the infundibular region due to some strongly irritative agent to which some of the cells gradually succumb.

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Some Aspects of the Pharmacology of Apomorphine Hydrochloride.

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From the Department of Physiological Chemistry and Pharmacology, the University of Chicago.

In spite of many chemical and pharmacological studies of apomorphine during the past 50 years, there still seems to be considerable doubt as to whether or not the green coloration which appears in apomorphine solutions is evidence of deterioration or of the formation of a new toxic substance. Guinard,[1] Eggleston and Hatcher[2] found little change pharmacologically in solutions of apomorphine which had become a deep green. Heinekamp,[3] however, believed that such apomorphine solutions were definitely less potent. The United States Pharmacopoeia X, and some text books (*e. g.,* Trendelenburg[4]) recommend that solutions should not be used, especially if green immediately or shortly after the dissolving of the solid drug. Harnack[5] declared that the oxidation with subsequent loss of potency occurred with some apomorphine preparations and not with others. The experiments reported below were, therefore, in part, undertaken to determine the effect of oxidation of the dissolved drug (as shown by the green color of the solution) upon its pharmacological characteristics.

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[1] Guinard, L., cited by R. Magnus, *Handb. d. exper. Pharm.*, II, pt. 1, 440.

[2] Eggleston, C., and Hatcher, R. A., *J. Phar. Exp. Ther.*, 1911-1912, iii, 551.

[3] Heinekamp, W. J. R., *J. Lab. Clin. Med.*, 1922, viii, 165.

[4] Trendelenburg, P., *Grundl. d. allg. u. spez. Arzneiverordnung,* Leipzig, 1926.

[5] Harnack, E., *Arch. exp. Path. Pharm.*, 1874, ii, 254.

Three different crystalline preparations* of apomorphine hydrochloride were used for making both the new and the old solutions which were injected into 7 healthy unanesthetized dogs of from 7 to 11 kilos for 1 to 4½ months. Four of the 7 animals were injected daily for at least 4 months. The procedure finally adopted was to inject the animals once daily. Fresh solutions and green aged solutions of a given sample of apomorphine were administered on alternate days, subcutaneously. The minimal effective dose of 0.15 to 0.20 mg. per kilo was found to be the same as that reported by Eggleston and Hatcher. The data in Table I are representative of the results with injections over a period of 3 or 4 months.

TABLE I.

Potency of Fresh and Old Solutions of the Same Apomorphine Hydrochloride.

Date	Animal	Apomorphine solution	Dose per kg.	Vomiting movements		Remarks
				First	No.	
				Minutes after injection		
Aug. 21, 1927	6	Fresh	0.15 mg.	9.5	1	Animals injected daily since May 18, 1927.
	7	"	" "	9.2	1	
Aug. 22, 1927	6	127 days old	" "	8.7	1	Solution black-green
	7	" " "	" "	8.6	1	
Aug. 23, 1927	6	Fresh	" "	8.6	1	
	7	"	" "	0.0	0	
Aug. 24, 1927	6	129 days old	" "	7.2	1	Solution black-green
	7	" " "	" "	8.7	2	

In green-black solutions at least 4½ months old and in one 12 months old, we found no pharmacological evidence of either quantitative or qualitative changes in the drug. The minimal effective dose was the same for the fresh solutions as for the old; the colored solutions caused no new or toxic effects. Injections made once daily into the dogs for 4 months produced neither hypersusceptibility (Richet[6]) nor tolerance to the drug.

Our experiments also indicated that, judged grossly, animals receiving a "nauseant" dose of apomorphine daily for 4 months exhibited no pre-injection salivation similar to that observed by Tatum and Collins[7] after 7 or 8 daily injections of morphine. Podkopaeff[8] has reported the establishment of a conditioned salivary reflex in dogs by numerous injections of apomorphine hydrochloride accompanied by the note of an organ pipe. It therefore seemed necessary to determine as definitely as possible whether or not apomorphine did resemble morphine in establishing a conditioned salivary reflex. Accordingly, fistulae of Warton's ducts were made in 2 dogs to permit of a more accurate measurement of the salivary secretion as was done by Kleitman and Crisler.[9] As before, however, the daily injection of apomorphine hydrochloride over an additional 3 months in a dose sufficient to provoke definite salivation, retching and vomiting never caused the appearance of a salivary conditioned reflex similar to that encountered after morphine injections. The data in Table II are typical and illustrate how the results were obtained.

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* Made by Kahlbaum, Merck and Schuchardt.

[6] Richet, C., *C. R. Soc. Biol.*, 1905, lviii, 955.

TABLE II.
Salivary Secretion after Morphine and Apomorphine Injected Subcutaneously.

Date	Animal	Secretion of saliva		Remarks
		10 minutes before injection	10 minutes after injection	
Dec. 11, 1927	6	6.0 cc.	8.0 cc.	30 mg. morphine hydrochloride given subcutaneously to each dog daily for 13 days previously.
	7	4.0 cc.	7.0 cc.	
Jan. 8, 1928	6	0.0 cc.	7.0 cc.	Daily injections of 0.2 mg. per kilo of apomorphine hydrochloride only, begun 18 days after last morphine hydrochloride injection.
1st day	7	0.0 cc.	10.0 cc.	
Feb. 15, 1928	6	0.0 cc.	3.8 cc.	Apomorphine hydrochloride injections continued daily.
39th day	7	0.0 cc.	3.2 cc.	
April 8, 1928	6	0.0 cc.	2.0 cc.	Last apomorphine hydrochloride injection.
92nd day	7	0.0 cc.	1.9 cc.	

The experiments of Henderson[10] and others indicate that apomorphine has no peripheral action but stimulates the salivary centers and, unlike morphine,[11] does so even when injected into animals under anesthesia. Apparently apomorphine induces salivation, not so much by producing a nausea centrally, as by stimulating the motor cells of the salivary nuclei. This is in agreement with the frequently expressed belief that apomorphine may cause vomiting with little nausea. In our animals, dogs given apomorphine soon after eating a meal, commonly eat the vomitus immediately. This would hardly be possible if the animals were truly nauseated.

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[7] Collins, K., and Tatum, A. L., *Am. J. Physiol.*, 1925, lxxiv, 14.

[8] Podkopaeff, N., cited in *Centralbl. ges. Neurol.*, 1925, xxxix, 214.

[9] Kleitman, N., and Crisler, G., *Am. J. Physiol.*, 1927, lxxix, 571.

[10] Henderson, V. E., *J. Phar. Exp. Ther.*, 1910, ii, 1.

[11] Kleitman, N., Personal communication.

Summary: 1. The development of a green color in old (1 week to 1 year) apomorphine hydrochloride solutions appears to be accompanied by no qualitative or quantitative changes in the pharmacological actions of the drug.

2. Dogs receiving an emetic dose of apomorphine daily for 4 months exhibit no tolerance or hypersusceptibility. *No nauseant conditioned salivary reflex similar to that of morphine appears.*

3979

Chloride and Water Excretion After Intraperitoneal Administration of Salt Solution.

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Since Blackfan and Maxcy[1] pointed out the advantages of administering saline solution intraperitoneally in dehydrated infants, the method has been widely adopted, and is regarded as a most valuable therapeutic measure If isotonic or slightly hypotonic solutions of saccharose are injected intraperitoneally in rabbits, in amounts of 100 ccm., a considerable diuresis ensues,[2] with an increased excretion of sugar. As a consequence we were interested in determining the output of water and chloride in acute experiments after similar injections of isotonic saline solution.

Male rabbits weighing between 2 and 2.5 kilos were used throughout the experiments, and were prepared according to methods previously outlined. Food was uniformly given as 200 gm. of carrots, 18 hours previous to each experiment. No additional water was given. A large soft rubber catheter a demeure was used to collect the urinary output. Specimens were collected during 10 minute periods for one hour or more, and at the middle of each 10 minute period 0.2 ccm. of blood was drawn from an ear vein

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* This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

[1] Blackfan and Maxcy, *Am. J. Dis. Child.*, 1918, xv, 19.

[2] Curtis, *Biochem. Z.*, 1925, clxiii, 109.

in a micropipette, for analysis. Chloride values were determined by a modification of the Bang method.

Sixty-one experiments, to determine the normal urinary output and blood chloride of 7 normal rabbits, demonstrate somewhat inconsistent results. In general the urinary output per 10 minute period is low, averaging about 0.2 ccm.—less often it was higher, averaging 1.6 ccm. Whether high or low it tended toward uniformity during the succeeding 10 minute periods. Minor individual differences were apparently associated with the different rabbits. The blood chloride remained fairly constant during each experiment.

Forty-eight similar experiments were then done on the same animals, with the addition of an intraperitoneal injection of 100 ccm. of isotonic sodium chloride solution at body temperature. The injections were uniformly preceded by 2 or more control 10 minute periods, and the urinary volume, chloride concentration, and blood chloride concentration were also determined for 6 or more 10 minute periods following the injection. The chloride concentration of the fluid injected was determined and also after its removal from the peritoneal cavity at the end of an hour. Occasionally no fluid could be obtained through the trochar at the end of an hour. Such a rapid and complete absorption was associated with a diuresis.

Subsequent to such an intraperitoneal administration of saline solution there is usually a slight but definite elevation of the blood chloride. The urinary output of chloride increases but slightly as a rule—in a few experiments, however, there occurred a marked increase. The urine volume as a rule increased but little. In experiments where the output during the control periods was high, there was even a decrease during the ensuing periods. Occasionally a frank diuresis followed. The experiments thus offer a rational basis for the administration of saline intraperitoneally in dehydration states since the large quantities of injected saline rarely cause a diuresis.

The peritoneal fluid removed at the end of an hour always coagulated spontaneously and had a high cell count. It may contain up to 1.5% of albuminous substance.[3] The high cell count is doubtless associated with an early inflammatory reaction of the peritoneum.[4] Occasionally fluid was not obtained for analysis at the end of an hour, otherwise a constant lowering of the chloride concentration of the fluid injected was noted.

Along with the evidence of the entrance of water and chloride into the blood stream, and eventually the tissues, and that of peritoneal irritation, another factor should be considered in evaluating the administration of saline intraperitoneally. If the delta, electric conductivity, chloride and urea concentrations of the fluid are determined at the end of an hour, calculations may be made which reveal the presence of achloride electrolytes and organic crystalloids in the fluid.[2] These have entered by permeability changes. Thus, while water and chloride enter the blood, other electrolytes and crystalloids are deviated from the blood into the injected fluid. What significance this deviation may have is problematic.

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[3] Curtis, *Biochem. Z.*, 1927, clxxxvi, 95.

[4] Denzer and Anderson, *Am. J. Dis. Child.*, 1921, xxi, 565.

3980

Autosterilizing Power of the Nasal Mucous Membrane.

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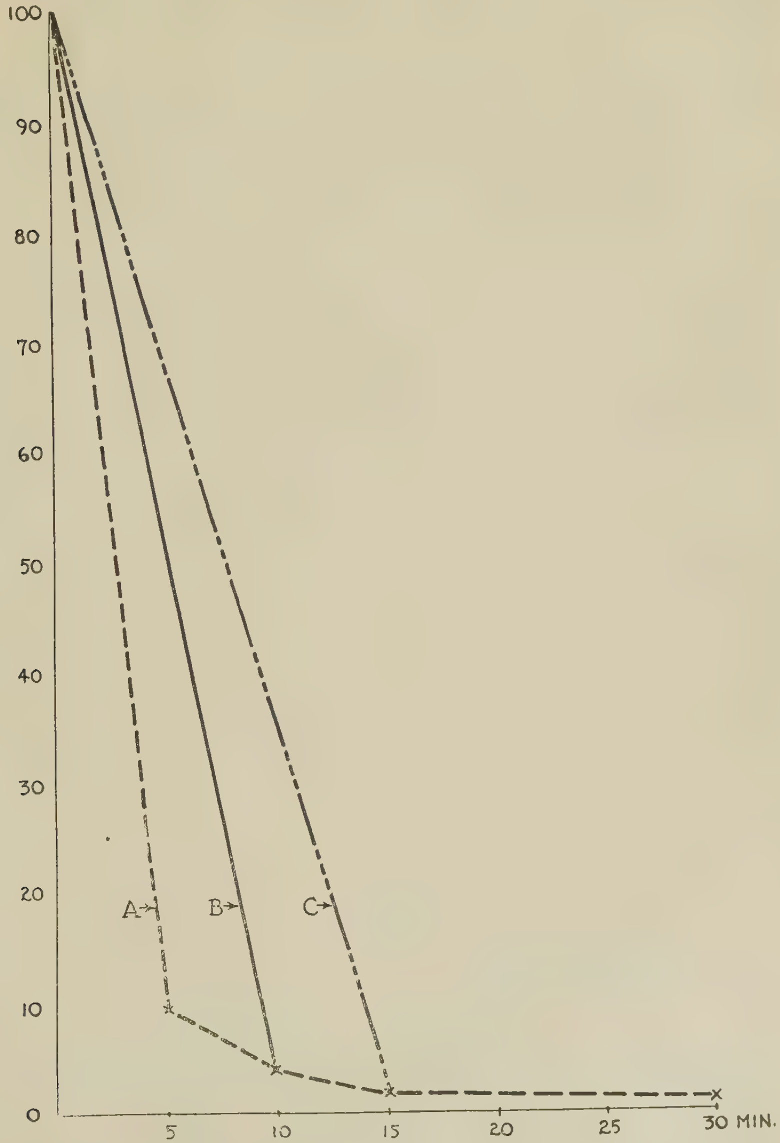
The average rate of disappearance of bacteria sprayed upon the mucous membrane of the nose has been determined for a series of animals, including guinea pigs, rabbits, dogs and humans. The technic finally used was the following: Broth cultures were diluted 1:200 with saline. A spray delivering 0.15 cc. (±0.01) was used to distribute the bacteria in each nares. A swab touched upon the surface of the respiratory portion of the nose at several places in a uniform manner and streaked well over the surface of an agar plate. This technic was repeated at intervals varying from one minute to one hour. An average of 400 experiments in normal humans, using 42 different subjects is shown in the accompanying graph. The average number of viable bacteria sprayed was 20,000,000. The swab taken immediately after spraying contained an average of 2,000 bacteria. After 5 minutes the number was reduced to about 50 colonies, 10 minutes about 15 colonies, after 15 minutes about 8 to 10 colonies, after 30 minutes about 5 to 10 colonies. At times none were present after this period. After one hour sometimes 1 or 2 colonies appeared, but ordinarily all of the sprayed bacteria were absent from such agar cultures. The same results were obtained with the other animals used by us. There were 185 animal experiments. If the same bacteria are sprayed in the same number in both sides of the nose, immediate swab only being obtained from one side, and after 5 minutes specimen collected from both nares, the rate of reduction in both sides being the same. This



GRAPH 1.

Average of 425 normal human experiments. Ordinate, % of bacteria in relation to the number found upon the nasal mucosa immediately after spraying. Abscissa, time in minutes after spraying bacteria into nose.

A—Swabs taken after 5, 10, 15 and 30 minutes.

B—Swabs taken after 10 minutes.

C—Swabs taken after 15 minutes.

Average rate of reduction is not influenced by mechanical removal of bacteria by swabbing nasal mucosa.

type of experiment has been carried out many times with several different time intervals. The mechanical removal of bacteria by swabbing the mucosa does not play a demonstrable rôle in this reaction.

Swabs taken from the posterior pharyngeal wall show that these bacteria are not removed to an appreciable extent by being passed into the throat from the nose. Immediately after spraying 20 millions of bacteria into each side of the nose there are none found in the throat in the majority of our experiments. After 10 minutes there are from 25 to 40 colonies upon an agar plate surface smeared with a swab taken from this region. There is a reduction in the number after this time. At the 30 or 45 minute interval few if any viable test organisms can be found in the throat.

B. prodigiosus and *B. coli* have been found most satisfactory for this work; both disappear in the same ratio. "S" and "R" types of *B. coli* are removed at the same rate. In isolated instances we have observed an "R" variant appearing on the agar cultures after spraying the "S" type of *B. coli.* In one experiment lysogenic coli colonies were observed after the "R" type was seeded on the nasal mucosa.

As many as 8 successive seedings of the same bacteria at 30 minute intervals were placed in the nose. There is no evidence of exhaustion of this disinfecting mechanism, in fact, the bacteria are removed at a more rapid rate upon successive application. This is probably due in great part to the mechanical irritation of repeated application of the swab to the mucous membrane.

Broth cultures have been made from one of the few colonies appearing upon an agar plate 15 to 30 minutes after the application of the bacteria to the nasal mucosa. These were sprayed into the nose in the same manner the following day. The same technic has been carried out for 9 successive days. There was no evidence of an acquired resistance on the part of these bacteria that had survived the maximum period of time.

Nasal washings, nasal secretions collected by packing the nose with dry sponges do not contain inhibiting substance for the same bacteria *in vitro* experiments. Such secretions, collected after a period of repeated spraying of the nose with bacteria, do not contain inhibiting substance in various dilutions in broth cultures. In fact all of our results demonstrate growth promoting substances in nasal secretions for the same bacteria that are rapidly rendered non-viable when placed upon the normal nasal mucosa.

Normal human subjects placed in a cold room (45° F., humidity 30%) do not differ in the self-disinfecting power of the nose as determined by our technic from that found in the ordinary room temperature (70° F., 70% humidity). The same subjects placed in hot rooms (95° F., and 90% humidity) show a retardation in the removal of sprayed bacteria. After 45 minutes to one hour the bacteria have disappeared but the curve is nearer a straight line. The rapid reduction of 90% of the inoculated dose within 5 minutes does not usually hold for the hot room environment.

This self-disinfecting power of the mucous membrane of the nose of healthy volunteers may play a major rôle in explaining the failure to seed the nose and throat with bacteria in influenza and other respiratory epidemiological studies.

Summary: 90 to 95% of the viable bacteria placed upon the mucous membrane of the nose are rendered non-viable within 5 to 10 minutes. Repeated application of bacteria to this mucosa is not associated with an exhaustion of this disinfecting mechanism. Cultures obtained from bacteria that have survived the initial period of disinfecting action are removed as promptly as the original strain from the nasal mucosa. Repeated applications did not lead to the development of a resistant strain.

"S" and "R" types of *B. coli* are removed at the same rate. Isolated instances of the appearance of an "R" variant from an "S" type of *B. coli* and also of lysogenic *B. coli* colonies were observed.

We were unable to demonstrate growth inhibiting substance in nasal washings and secretions *in vitro*.

Subjects in warm rooms showed a retardation in the disappearance of the bacteria from the nasal mucosa.

3981

Influence of Local Application of Fresh Animal Serum, "Complement," on Acute and Chronic Gonorrhoeal Infections.

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Human serum has no complement for gonococci (Scaltritti[1]). This fact gives us a new viewpoint in the treatment of gonorrhoeal and blenorrhoeal conditions. With the expectation of a mild reaction I (Pribram) tried this treatment in a 4-year-old girl who had a gonorrhoic infection for 4 weeks. I introduced a few drops of a fresh animal serum into the urethra and the vestibulum of the vagina. The discharge became abundant the next day. The microscopic inspection revealed pus cells filled with gonococci. I stopped the treatment in this case and continued with an argyrol salve in the usual way. The first attempt showed that we are able to induce an acute exacerbation of a subchronic gonorrhoeal process by means of the application of fresh animal serum. Besides this it became evident that the serum had a strong chemotactic action on human phagocytes and increased their opsonic index.

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[1] Scaltritti, A., *Annales de l'Institut Pasteur*, 1925, xxxix, 865.

The further investigations were carried on by Dr. Jonas on male adults with acute and chronic gonorrhoeal affections. The method consisted in a very cautious instillation of a few cubic centimeters of fresh animal serum into the urethra. The serum was allowed to remain in the urethra for a few minutes. The discharge, absent or very poor in chronic cases, became abundant and showed many phagocytized gonococci. It disappeared after 1 to 3 days almost completely. We had the opportunity to treat 2 patients with a fresh, hitherto untreated gonorrhoea. No other treatment than the local application of fresh animal serum on 5 successive days was instituted in this case. The abundant discharge of phagocytosis following the treatment stopped completely after the instillation of the serum had been discontinued.

Further studies will be necessary in order to state exact quantities of the complement which have to be used, furthermore the most effective serum species must be determined and the number of instillations which are necessary for a complete cure. This effect can only be expected in fresh uncomplicated gonorrhoeal conditions, because the bacteriolytic action is localized and cannot have any effect where the serum has no direct contact with the microorganisms. The phagocytosis depends also on the presence of the serum. Our treatment has been performed without the addition of an antigonococcic serum as yet and it is very probable that the effect can be improved and the quantity of the complement can be decreased by the application of a bacteriolytic serum activated by a suitable complement.

The fact that complement for certain human bacterial infections is present in fresh animal serum but absent in human serum may prone to be an initiative for an attempt for a "complement treatment" of different local bacterial infections. Working along this line will bring us to the consideration of the treatment of the blenorrhoea of the eye with a similar method. I further started with analogous investigations in other diseases, especially in those infections in which a phagocytosis is desirable.

Minnesota Branch.

University of Minnesota Medical School, April 25, 1928.

3982

Relationship Between Fibrin Content and Sedimentation Index in Normal Individuals.

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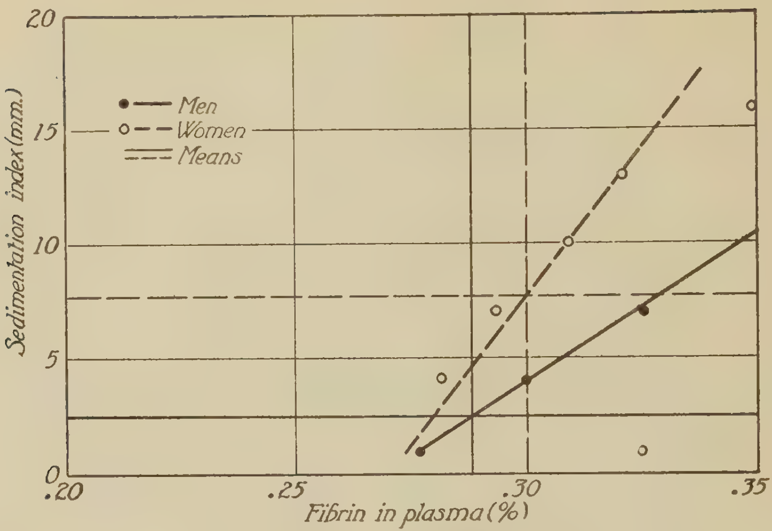
The series of investigations was carried out on normal individuals between 18 and 30 years of age. It includes 52 women and 50 men. The sedimentation index was determined by the Cutler method and the fibrin by the Wu colorimetric method, with slight modifications. The fibrin determinations were done in duplicate.

The average sedimentation index for women was 7.635 (mm.) ± .345; the average index for men was 2.380±.164. The difference between the 2 sexes is 5.255±.382. This difference is 16.3 times as large as its probable error, and is probably significant. The average fibrin content for women was .300 (gm. per 100 cc. plasma) ±.004. For men it was .288±.004. The difference between the 2 sexes is .012±.006. Since this difference is only twice as large as its probable error, it cannot be considered certainly significant.

Treatment of the sedimentation indices and the fibrin contents by the statistical method gave a correlation coefficient of .312±.084 for the women. This is 3.83 times as large as its probable error and is probably significant. The empirical mean fibrin values as ordinates are plotted against the sedimentation indices in the graph. While certain of these means, particularly that for the fibrin content of .325 (index 1), deviate rather widely from the regression line, it must be remembered that the total number of cases is 52, and that certain of these means are based on very few analyses.

The correlation coefficient for men was .328±.085, which is 3.86 times as large as its probable error, and is probably significant. The empirical mean fibrin values plotted against the sedimentation indices, and the fitted regression line is shown in the graph.



For women $F = .27099 + .0038S$
For men $F = .26984 + .00763S$

FIG. 1.

The difference between the correlation coefficients for the 2 sexes is only .016±.120. Since the difference is less than its probable error, it cannot be considered significant.

3983

Interrelationship of Certain Physico-Chemical Constants of Plant Saps.*

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Increasing attention is being paid to the physico-chemical properties of plant saps. Earlier studies were devoted almost exclusively to the problems of the osmotic concentration of the plant saps, particularly as related to environmental conditions. Dixon and Atkins[1] pointed out that in order to secure uniform samples of sap the plant tissues must be made permeable by freezing. They used liquid air for freezing the material, and later Gortner and Harris[2] modified this method by using a simple salt and ice bath. Since that time a voluminous literature has accumulated[3] in regard to the influence of geographical environment and climate on the osmotic properties of plant saps. In most of the work carried out by Harris, and Harris and Gortner the electrical conductivity of the tissue fluids has been recorded, in addition to osmotic concentration, but it is only recently that a new factor has been shown to be of importance.

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* Published with the approval of the Director, as Paper No. 780, Journal Series, Minnesota Agricultural Experiment Station.

[1] Dixon, H. H., and Atkins, W. R. G., *Sci. Proc. Roy. Dublin Soc.*, (n. s.) 1913, xiii, 422.

Newton and Gortner[4] devised a technic for the measurement of "bound water" *i. e.,* the water which appears to be associated with hydrophilic colloids and consequently is differentiated from the free water which is free to dissolve organic and inorganic solutes. Newton[5, 6, 7] has demonstrated that a measurement of bound water content is of great importance in the study of winter hardiness of plants, and in similar unpublished work Newton has likewise demonstrated that measurements of bound water are of importance in studying problems of drought resistance. Robinson[8, 9] showed bound water to be a factor in ecological problems of insects. Gortner[10] has called attention to some of the relationships involving free and bound water as affecting biological problems.

Recently Meyer[11] has made a study of the physico-chemical properties of plant saps from a series of mesophytic plants from Ohio. He records the % of water in the fresh leaves, the amount of sap yielded by 100 gm. of fresh leaves under a pressure of 5,000 pounds to the square inch, the % of total water expressed from the leaves, the osmotic concentration of the expressed sap, the total solids in the expressed sap, and the bound water in the expressed sap determined by the method of Newton and Gortner.

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[2] Gortner, R. A., and Harris, J. A., *Plant World,* 1914, xvii, 49.

[3] For representative publications see the bibliography in the paper by Harris, J. A., Gortner, R. A., *et al., J. Agr. Res.,* 1924, xxvii, 893.

[4] Newton, R., and Gortner, R. A., *Bot. Gaz.,* 1922, lxxiv, 442.

[5] Newton, R., *J. Agr. Sci.,* 1922, xii, 1.

[6] Newton R., *J. Agr. Sci.,* 1924, xiv, 178.

[7] Newton, R., University of Alberta, Coll. of Agr. Research Bull. No. 1, 1923, p. 53.

[8] Robinson, William, *J. Econ. Entomol.,* 1927, xx, 80.

[9] Robinson, William, Colloid Symposium Monograph, Vol. V, Chemical Catalog Co., N. Y., 1928, p. 199.

[10] Gortner, R. A., "Adsorption and Vital Phenomena," in Mayo Foundation Lectures on Biologic Aspects of Colloidal and Physiologic Chemistry, Saunders and Co., Philadelphia, 1927, p. 133.

[11] Meyer, B. S., *Ohio J. of Sci.,* 1927, xxvii, 263.

Inasmuch as there are but few data on bound-water determinations and most of these data are on a limited number of plant types, it seemed worth while to ascertain whether or not there was any direct relationship between the bound-water content of the plant saps studied by Meyer and any of the other values which he records.

We have accordingly calculated the coefficients of correlation, using the formula suggested by Harris,[12] for all of the various combinations possible from Meyer's data. In some instances, one or another of the values was lacking, which explains why the "number of determinations" varies from set to set. We have likewise calculated the coefficients of correlation for the ligneous forms and for the herbaceous forms separately, inasmuch as Harris, Gortner, Lawrence, *et al*[13, 14, 15] have shown that ligneous forms may differ markedly from herbaceous forms in regard to the magnitude and relationship of the physico-chemical constants of the plant saps.

Table I shows the coefficients of correlation which were obtained between the various physico-chemical constants of the plant saps, as listed in Table I of the paper by Meyer.[11] It is unnecessary to discuss this table at length.

It will be noted that there is no significant relationship between the osmotic pressure and bound water, although in the ligneous forms there appears to be a slight tendency toward an increased bound-water content with increased osmotic pressure. However, this is less than twice its probable error, and in the herbaceous forms the reverse relationship occurs, although here again the coefficient is only slightly more than twice its probable error. Combining both forms, there is no appreciable correlation.

Essentially the same relationships hold when we consider the total solids in the sap, and bound water, a positive correlation in the ligneous forms which is barely more than its probable error, a slightly larger negative relationship in the herbaceous forms, and a positive correlation nearly 3 times its probable error when both forms are combined.

When we consider the total solids in the sap, and the osmotic pressure, we find, as one would expect, a high correlation. Inasmuch as the osmotic pressure is due to dissolved solutes, one should find a perfect correlation, providing all of the dissolved solutes were contributing to the osmotic pressure. The correlation in this case is considerably higher for ligneous forms than for the herbaceous forms.

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[12] Harris, J. A., Am. Nat., 1910, xliv, 693.

[13] Harris, J. A., Gortner, R. A., and Lawrence, J. V., *J. Phys. Chem.*, 1921, xxv, 122.

[14] Harris, J. A., Lawrence, J. V., and Gortner, R. A., *Physiol. Res.*, 1916, ii, 1.

[15] Harris, J. A., Gortner, R. A., Hoffman, W. F., Lawrence, J. V., and Valentine, A. T., *J. Agr. Res.*, 1924, xxvii, 893.

TABLE I.

The coefficients of correlation between certain physico-chemical constants of plant saps.

	Ligneous forms		Herbaceous forms		Both ligneous and herbaceous forms	
	No. of determinations	Coefficient of correlation	No. of determinations	Coefficient of correlation	No. of determinations	Coefficient of correlation
Osmotic pressure and bound water	23	+0.227±0.133	15	—0.345±0.153	38	+0.172±0.106
Total solids in sap, and bound water	23	+0.175±0.136	14	—0.289±0.165	37	+0.274±0.103
Total solids in sap, and osmotic pressure	25	+0.806±0.047	14	+0.546±0.127	39	+0.781±0.042
% of total water expressed and bound water	22	+0.373±0.124	10	—0.322±0.191	32	—0.013±0.119
% of total water expressed and total solids in sap	24	+0.044±0.137	10	—0.044±0.213	34	—0.207±0.111
% of total water expressed and osmotic pressure.	25	—0.144±0.132	10	+0.319±0.191	35	—0.176±0.110
% of total water expressed and moisture content of leaves	31	+0.482±0.093	11	—0.658±0.115	42	+0.423±0.085
Water content of leaves and bound water	22	+0.318±0.129	15	+0.268±0.162	37	+0.011±0.111
Water content of leaves and osmotic pressure	25	—0.216±0.129	15	—0.703±0.088	40	—0.600±0.068
Water content of leaves and total solids in sap	24	—0.379±0.118	14	—0.732±0.084	38	—0.672±0.060

The relationship between % of total water expressed and bound water yields a positive correlation approximately 3 times its probable error for ligneous forms, a negative correlation slightly more than twice its probable error for the herbaceous forms, and with both forms combined a correlation of essentially no value. It would appear that in this case, as the amount of total water expressed increases, the bound water increases for the ligneous forms, indicating that the true protoplasmic contents may increase in the last portions of water from the ligneous plants, whereas the first portions of water expressed from the herbaceous plants may be richer in the true protoplasmic contents. In any event, the 2 forms are sharply differentiated by this comparison.

Considering the % of total water expressed in relation to the total solids in the sap, we find no significant correlation for either ligneous or herbaceous forms, although for both forms combined we have a negative correlation approximately twice its probable error.

For % of total water expressed and osmotic pressure there is a negative correlation for the ligneous forms which is not significant, a positive correlation nearly twice its probable error for herbaceous forms, and a slight negative correlation, probably statistically insignificant, for both forms combined.

For the percentage of total water expressed and the original moisture content of the leaves there is a significant positive correlation for the ligneous forms, a somewhat higher negative correlation for the herbaceous forms, and a significant positive correlation for both forms combined. These values indicate that the percentage of total water expressed increases with the increase of the moisture content of the leaves for ligneous forms, but decreases with increased moisture content of the leaves for herbaceous forms. This is rather surprising, and further data are desirable on this point.

For the relationship of the water content of the leaves and bound water we have in all cases a slight positive correlation, indicating that as the water content of the leaves increases, there is a tendency for the bound water to increase. In the case of the ligneous and the herbaceous forms separately the correlation is less than 3 times the probable error. For both forms together the correlation becomes insignificant.

For the water content of the leaves and the osmotic pressure of the expressed sap, there is a slight negative correlation, which is probably not significant, in the case of the ligneous forms, the correlation being less than twice its probable error. In the case of the herbaceous forms, however, there is a large negative correlation, and for both forms together a significant negative correlation. This indicates that in the herbaceous forms an increase of moisture content in the leaves causes a marked decrease in osmotic pressure, whereas for the ligneous forms the decrease, if it occurs, is relatively slight.

For the water content of the leaves and the total solids in the sap we have again a negative correlation, both for the ligneous and for the herbaceous forms, as well as for the 2 forms combined. Here again, the ligneous forms show a much lower correlation than do the herbaceous forms, indicating that physiologically the 2 forms differ to a very marked extent.

It will be noted that in none of the series in which bound water is a factor do we find high coefficients of correlation. This justifies the labor necessary for making these calculations, inasmuch as it demonstrates that the measurement of bound water in a plant sap is the measurement of a factor which is essentially independent of the properties which have previously been measured, and that the bound-water values cannot be obtained from any of the ordinary measurements. Consequently these data can be taken as indicating that measurements of bound water may prove helpful in studying the properties of plant saps as related to geographical distribution.

Another point brought out by the table has already been referred to, namely, the marked differences which are shown in certain of the relationships, when one compares ligneous with herbaceous plant forms. It should be obvious that there is a marked difference in the physico-chemical properties of the plant saps of these two forms, as has already been pointed out.[13, 14, 15]

3984

Improved Method for the Extraction of Melanin from Human Urine.

GRACE MEDES AND HILDING BERGLUND.

From the Medical Service of the University Hospital, Minneapolis.

The futility in expecting to obtain an unaltered metabolic product of a complex character like human or animal melanin after prolonged boiling with caustic or concentrated mineral acids, was pointed out by Gortner[1]. The method of isolation of urinary melanin by direct precipitation of the urine either with basic lead acetate or with barium hydroxide suffers from the disadvantage that a great variety of compounds besides the melanin is taken down by either method; which fact does not facilitate the purification.

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[1] Gortner, R. A., *J. Biol. Chem.*, 1910, viii, 341.

Due to the great scarcity of melanuria in cases of melanosarcoma in man, the problem of a simple and at the same time mild method for the isolation of melanin from human urine is only seldom attacked. The fact that we are engaged in the isolation of another cyclic urinary compound[2] made it possible for us to use the occasion of obtaining melanotic urine from a middle aged man with multiple metastases from a melanosarcoma of the skin. The abundance of melanin produced and circulated in the organism in this patient was demonstrated not only by the occurrence of melanin in his urine but also by a unique diffuse melanotic discoloration of the skin giving a slate colored character particularly to face and hands.

For the isolation of the melanin the following procedure has been employed: The urine is evaporated on a water bath in a current of air and the residue is extracted with absolute methyl alcohol. The extraction is carried out in a tall cylinder or beaker and the salts allowed to settle slowly. This process is repeated several times with fresh washings of methyl alcohol until the final washing is straw colored or colorless. This extracts a variety of substances leaving the bulk of the salts as well as all the melanin. The same procedure is then carried out with methyl alcohol acidified slightly with HCl, about 2 drops of concentrated HCl per 100 cc. of absolute methyl alcohol. This extracts the melanin. The acidified methyl alcohol from repeated washings is united, the melanin precipitated by the addition of ether, the solutions centrifuged and the supernatant liquid decanted. The precipitate, which consists of almost pure melanin, is redissolved in acidified methyl alcohol and reprecipitated with ether. The same process is repeated a second time. The precipitate is now washed several times with water, then with ethyl alcohol, finally with ether and dried in a current of dry air.

The product obtained is a dark brown or black powder, having the properties ascribed to melanin. It is insoluble in water, in neutral methyl alcohol, neutral ethyl alcohol and ether. It is slightly soluble in dilute acids, completely soluble in concentrated mineral acids and in alkali. It is somewhat soluble in alkaline methyl alcohol and highly soluble in acidified methyl alcohol. It does not reduce phosphomolybdic acid. It may be precipitated from the urine by dialysis, or carried down by saturating the urine with ammonium sulfate.

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[2] Medes, Grace, Berglund, Hilding, and Lohmann, Anne, PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 210.

It does not give the biuret reaction or the Hopkins-Cole reaction. Neither does it give a positive Millon's reaction. This corresponds to the observation of Gortner's (personal communication) that the melanin obtained from black wool of sheep without application of strong reagents gives a negative Millon's before hydrolysis.

The yield of melanin in our patient amounts to about 500 mg. per 24 hours.

3985

Experimental Tularemia in Birds.

R. G. GREEN AND E. M. WADE.

From the Department of Bacteriology and Immunology, University of Minnesota, and the Division of Preventable Diseases, Minnesota State Board of Health.

It has previously been reported that grouse are susceptible to experimental tularemia.[1, 2] Inoculation of an abrasion through the skin of a ruffed grouse results in a fatal septicemia.

Preliminary experiments which have been carried out on the susceptibility of the Hungarian partridge to experimental tularemia, indicate that this bird is highly susceptible to the disease, a fatal infection resulting from an open inoculation of a skin abrasion.

The pigeon appears to be relatively more resistant. In this bird an intramuscular injection causes the formation of a local lesion, and in some cases this is followed by a generalized invasion by the organism. Even with the production of a septicemia, no clinical symptoms have been observed in the pigeons in our series and those not killed have recovered.

The ring-necked pheasant also appears to be relatively resistant to experimental tularemia.

The domestic chicken appears to be absolutely resistant to the disease. Organisms injected intramuscularly do not appear to invade and produce a general infection.

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[1] Parker, R. R., and Spencer, R. R., Sixth Biennial Report, Montana State Bd. of Entomology, 1925-1926, p. 30.

[2] Green, R. G., and Wade, E. M., PROC. SOC. EXP. BIOL. AND MED., in press.

3986

Growth of Long-bones of Human Fetus as Illustrated by the Tibia.

MEREDITH B. HESDORFFER AND RICHARD E. SCAMMON.
From the Department of Anatomy, University of Minnesota.

The growth in length of the human tibia in the latter part of prenatal life has been studied quantitatively from observations, made chiefly by Corrado[1] and by Tamassia,[2] on a series of 152 fetuses. The data include the total body-length, the total length of the tibia, the length of the tibial diaphysis, and the combined lengths of the superior and inferior (cartilagenous) tibial epiphyses. Regression formulae have been computed for certain relationships between these values and are given, with other data, in Table I. Fig. 1 shows the distributions of these observations and their regressive lines.

These formulae indicate that, in the period under consideration, the growth in length of the tibia and the lineal growth of its various

TABLE I.
Correlations and Empirical Formulae for the Human Fetal Tibia and Total Body-Length.

Dimensions compared	Sex and number of cases	Coefficients of correlation	Regression formulae	Root mean square deviations of observed from computed values (mm.)
Length of proximal diaphysis (X) and length of both epiphyses (Y)	Males (73)	0.5296±0.058	$Y=0.1660X+7.285$ mm.	2.10
	Females (49)	0.5438±0.068	$Y=0.1335X+8.991$ mm.	2.10
Total body-length (X) and length of diaphyses (Y)	Males (73)	0.9494±0.008	$Y=0.1384X-5.772$ mm.	2.50
	Females (49)	0.9617±0.007	$Y=0.1543X-13.650$ mm.	2.45
Total body-length (X) and length of both epiphyses (Y)	Males (73)	0.5676±0.005	$Y=0.0249X+5.850$ mm.	2.04
	Females (49)	0.5835±0.064	$Y=0.0230+6.521$ mm.	2.03
Total body-length (X) and total length of tibia (Y)	Males (73)	0.9556±0.007	$Y=0.1671X-2.019$ mm.	2.81
	Females (49)	0.9655±0.008	$Y=0.1743X-5.443$ mm.	2.64

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[1] Corrado, *Gior. d. Ass. Napol. d. Med. e. Natural.*, 1899, ix, 220.

[2] Tamassia, *Atti. R. Inst. Veneto d. Sc., Lett. e. Arti.*, 1893, 7 ser., iv, 815.

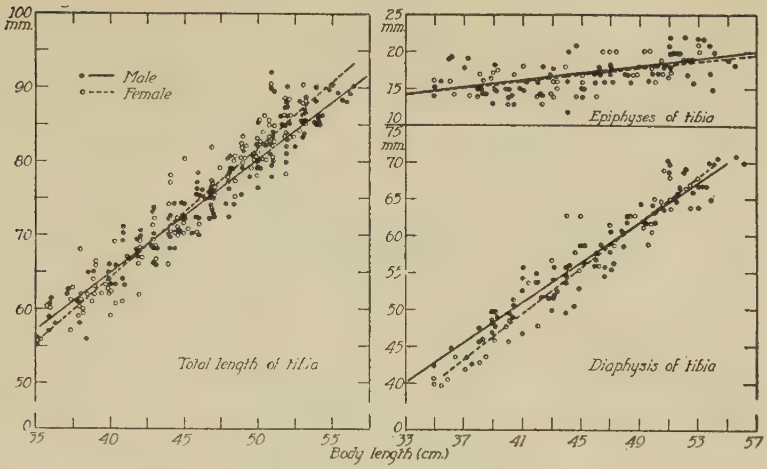

FIG. 1.

Field-graphs and regression lines representing the relation of the length of the tibia and its parts to the total body-length in the latter part of the fetal period.

parts are directly proportional to the growth in total or crown-heel length. The empirical formulae for the total length of the tibia and for the length of the tibial diaphysis, with respect to total body-length, are characterized by negative second constants, as are the various empirical formulae which have been developed for the external dimensions of the lower extremities (Calkins and Scammon[3]). The empirical formulae for the epiphyses, on the other hand, show positive second constants. Therefore, while the lengths of the tibia as a whole and of its diaphysis are becoming *relatively*, as well as absolutely, greater, the epiphyses are becoming *relatively* shorter, although absolutely longer.

Fig. 2 shows the calculated growth in length of the tibia and its several parts in the last trimester of prenatal life. The relation of the lengths of these structures to age, in fetal or lunar months, has been computed by the use of the empirical formula of Scammon and Calkins[4] for the relation between time and total body-length in the fetal period.

Differentiating the expressions for the lengths of the tibial structures with respect to time in the fetal period, we find that all parts of the bone decline in absolute rates of lineal growth in the latter part of prenatal life. The rate of absolute lineal growth of the tibia as a whole drops from 7.7 mm. per month at 7 lunar months to 6.4 mm. per month at 10 lunar months or birth. The

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[3] Calkins and Scammon, PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 353.

[4] Scammon and Calkins, PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 353.

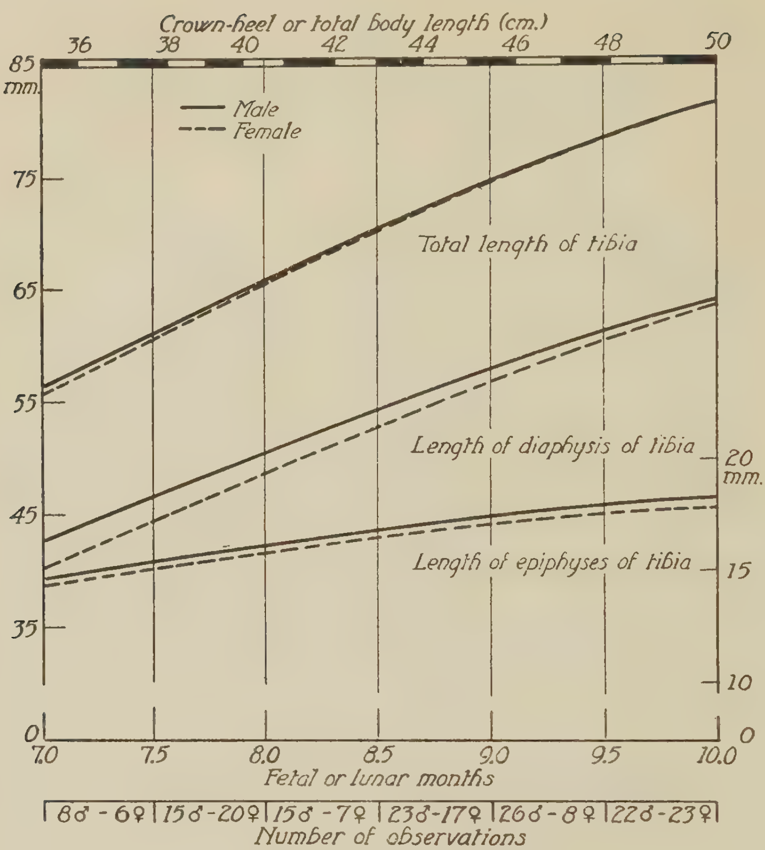

FIG. 2.

Curves, drawn from computed values, showing the growth in length of the tibia, the length of the tibial diphysis and the combined lengths of the tibial diaphyses in the last 3 months of prenatal life.

rate of diaphyseal growth declines from 8.6 mm. per month at 7 lunar months to 7.1 mm. per month at birth, and the rate of lineal growth of both epiphyses drops in the same period from 1.4 mm. per month to 1.1 mm. per month. These figures are based upon formulae computed by the method of least squares, using individual cases of both sexes.

Relative velocity ratios were obtained by dividing the absolute velocities of lineal growth of the several tibial parts at various times by the attained lengths of these times. These ratios decline between one-third and one-half between 7 lunar months and birth.

Ossification takes place in the superior tibial epiphysis in the 9th and 10th lunar months,[5] but the present data show no evidence that the appearance of this ossification center affects in any way the rate of growth of the epiphyses, either with respect to body-length or with respect to age.

Although the tibia is said to ossify earlier in females than in males,[5, 6] we find no significant sex differences in the rates of growth of the bone as a whole or in any of its parts, in the period under consideration.

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[5] Adair and Scammon, *Am. J. Obstet. and Gynecol.*, 1921, ii, 3.

[6] Pryor, *Anat. Rec.*, 1923, xxv, 257.

New York Meeting.

Yale University, May 19, 1928.

3987

Difference in Susceptibility of Mice and Rats to Experimental Production of Amyloidosis.

BALDUIN LUCKE AND L. A. MARKLEY.

From the Laboratory of Pathology, School of Medicine, University of Pennsylvania.

The chemical nature of amyloid, as well as the mechanism of its formation, has been very obscure. Experimental investigation of the problem has until recently encountered the difficulty that amyloid could not regularly be produced in animals. Thanks to the work of Kuczinski,[1] Jaffé,[2] Letterer[3] and others, this difficulty no longer exists. By parenteral injection of sodium caseinate and various other proteins and non-protein substances, as well as by feeding excessive amounts of protein, amyloidosis is produced *in mice* in the majority of those animals. In the course of the work of these investigators, certain observations have been made that greatly increase the interest of the problem of amyloid disease.

In experimental amyloidosis as well as in the spontaneous condition as it occurs in man, excessive breakdown of tissue proteins is known to take place. There is evidence that this is followed by an increased production of serum globulin, which apparently acts as the mother substance of amyloid.[3] It has been stated that on further injection of casein or other substances employed, globulin is precipitated in the tissues in altered form and that the resulting deposits constitute amyloid. The well recognized close relation of globulin to antibodies suggests that the whole process of amyloid formation is akin to an immunological reaction. This suggestion is at least worth testing, since, if true, experimental production of amyloid would be a most useful method in studying the mechanism of certain immunological reactions, having the unique advantage of producing easily recognizable and characteristic morphological changes.

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[1] Kuczniski, M. H., *Virchow's Arch.*, 1922, ccxxxix, 185; *Klin. Wochenschr.*, 1923, ii, 727, 2193.

[2] Jaffé, R. H., *Arch. of Path. and Lab. Med.*, 1926, i, 25; 1926, ii, 149.

[3] Letterer, E., *Beiträge zur path. anat. u. z. allg. Path.*, 1926, lccv, 486.

The best line of attack appears to be study of changes in blood chemistry during the course of production of amyloidosis. For this purpose an animal larger than the mouse is highly desirable. Guinea pigs and rabbits have, in the hands of other workers, given negative results. The rat was, therefore, selected as a possibly suitable animal.

Sixty adult mice and 16 rats were given daily intramuscular injections of pure casein (Hammersten), in 5% concentration with the addition of sufficient NaOH to give a clear solution. Since alkali caseinates may be denatured by heat sterilization and since it had not been determined whether denaturization affects its amyloid producing properties, different groups of animals received casein that had been prepared by boiling, or by autoclaving, or merely by addition of thymol. However, no constant difference in the effects of these various preparations was found. The dose given the mice was 15 mg. (0.3 cc.) in the gluteal region, while the rats received 15 mg. per 50 gm. of body weight for the first 35 injections and thereafter the same amount per 20 gm. body weight (*i. e.*, the same dose per body weight as was used in the mouse). The highest number of injections made was 91. The animals were kept on a constant diet of cracked corn and other cereals, fresh vegetables and bread (no additional water was given).

Animals were killed at intervals of 5 to 10 days. The spleen, liver, and kidneys were fixed in alcohol and in formalin. Paraffin sections were stained with haematoxylin and eosin and with cresyl-violet, the latter giving the clearest metachromatic reaction. Frozen sections were used for the iodine reaction and for specific staining with congo-red. Bielschowski's method was occasionally used to bring out the reticulum of the spleen.

A noteworthy difference was found in the effect of injections on the health of rats and mice. The former gave no evidence of illness, increased normally in weight and all survived to the end of the experiment. The mice, on the other hand, became emaciated and obviously ill. 29 out of 60 died during the experiment, but at autopsy no lesions (including suppuration) were found; these animals were not included in the study.

An equally striking difference was found in the tissues of the 2 species. No amyloid was found in any of the rats. 3 rats had received 20 to 34 doses, and 10 had received from 35 to 91.

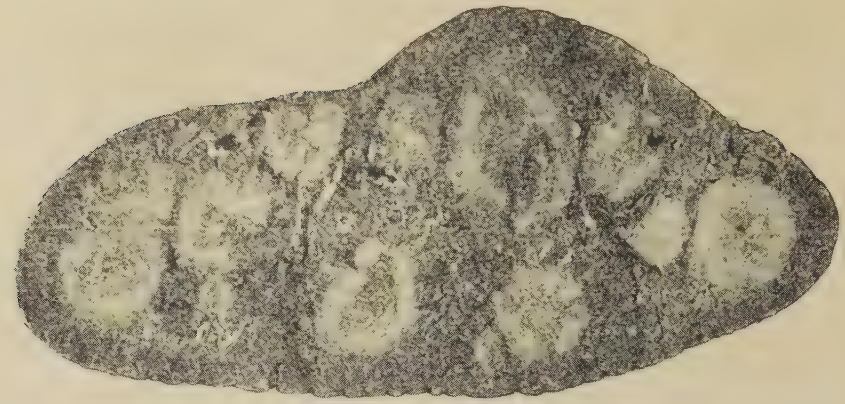

FIG. 1.
Amyloid infiltration of the splenic follicles. The mouse had received 25 injections of casein solution. (Magnification X20.)

The mice, on the contrary, showed positive results in 75% of 25 animals that survived for 20 or more injections. The deposits appeared first and were always more marked in the spleen, the liver showed moderate amounts, while in the kidneys no amyloid or traces only were found. The various characteristic microchemical reactions for amyloid were positive whenever the material was found. In the spleen the earliest deposits had a fibrillar or shred-like appearance coating the fibrils and capillaries in the outer rim of the follicles. After 25 or 30 injections all but the inner third of the follicles were generally involved (Fig. 1), the deposition then was nearly homogenous, but remnants of lymphoid tissue were present in a few places. The central arterioles showed typical involvement in about one-half of the follicles. The splenic pulp never contained any amyloid. In the liver the deposition occurred first in the walls of hepatic arteries, then in the branches of the portal veins and in the intralobular capillaries, especially those near the lobular peripheries. The amyloid was found between the capillary endothelium and the cords of liver cells, eventually compressing both and occluding the capillary (Fig. 2). The character of the deposits was somewhat different from those in the spleen, for nearly all of them consisted of delicate needles with the points directed towards the cords of liver cells. After complete occlusion of the capillaries the crystals appeared as spherical masses (Fig. 3). In a few kidneys traces only of amyloid were found, occurring in the glomerular loops and in the fibrous stroma.

It has been seen that under identical conditions of experimentation amyloidosis was produced in 75% of mice, but that entirely

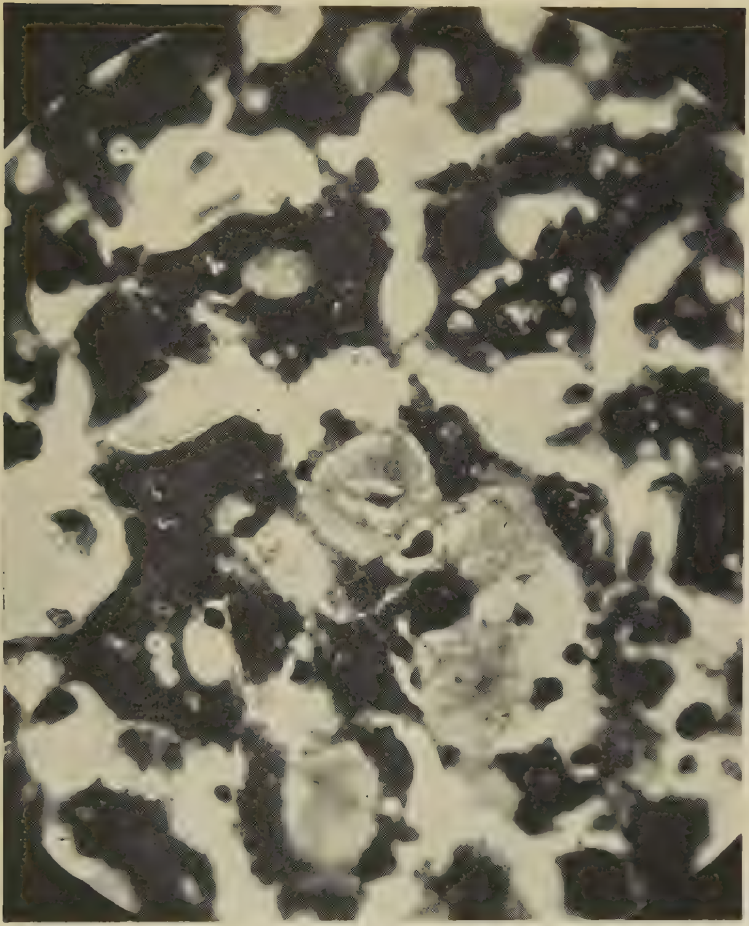

FIG. 2.

Amyloid infiltration of the liver. The amyloid occurs here in the form of crystalline needle-shaped deposits which surround and compress the sinusoids. An incompletely obliterated sinusoid, cut in transverse section, is seen as a dark spot in the center of one of the deposits. The mouse had received 25 injections of casein solution. (Magnification X920.)

negative results were obtained in the rat, a related species. Also that the mice suffered in health from the injections, while the rats thrived. It seems probable that in the latter, breaking down of tissue proteins is not sufficiently severe to lead to amyloid formation. No explanation of this difference in species susceptibility is known. That such difference exists is not, however, surprising since it is known that in man even individual susceptibility varies widely, in that comparable chronic suppurative conditions may or may not lead to amyloidosis.

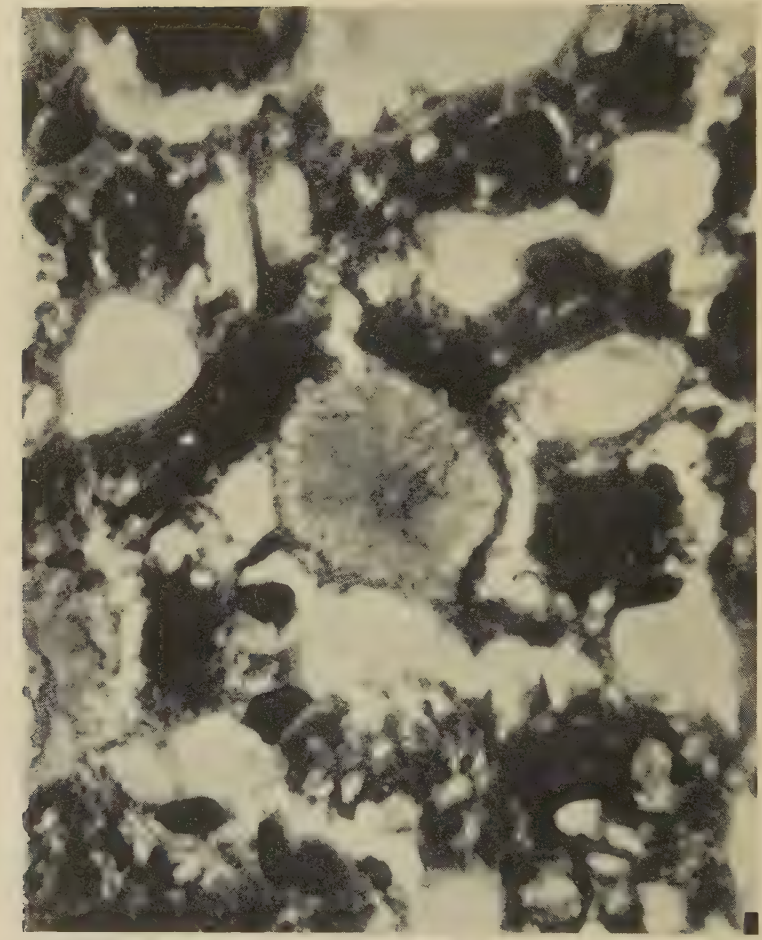

FIG. 3.
A higher magnification (X1840) of preceding photograph showing more clearly the crystalline structure of the amyloid, and the obliteration of the sinusoid.

3988

Histologic Effects of a Cholesterol-Free Diet on Adult White Rats.

W. M. BALDWIN.

From Albany Medical College.

A series of white rats was raised upon a cholesterol-free diet over a period of 3 generations since the question had arisen as to whether or not cholesterol was synthesized in these rats in the absence of sterols. The chemical end of the research was conducted by Knudson and Randles[1] and the experimental evidence ascertained seemed to justify their conclusion that this chemical was, to a certain extent, built up by the rat body.[2] At the conclusion of their experiments, the rats were killed and the tissues fixed in Zenker, Bouin, Meves, and formalin, and stained with a variety of the usual laboratory stains, chief among which was a combination of hematoxylin and Mercurochrome-220 Soluble (Hynson, Westcott and Dunning, Baltimore, Md.).

These rats of the third generation were weak, small, and undernourished, and lived only from 11 to 17 weeks after birth, *i. e.*, from 7 to 13 weeks after weaning. At the time of weaning they weighed from 26.0 to 30.0 gm., and at the time of death from 74.0 to 80.0 gm.

There was no evidence at the time of autopsy of either inflammation or degeneration in any of the body tissues. All of the body organs were reduced in size and in weight but preserved a fair normal proportion to the mass of the body in comparison to the controls.

All of the tissues and organs of the rats were studied in detail histologically. It was evident that the diminutive size of the various viscera had been attained less by a reduction in number of the individual functional units composing them, than by a decrease in the size of the cells forming these units. Hence, the tissues and organs presented the appearance of structural compactness, without a conspicuous absence of the characteristic cells ordinarily composing them.

An examination of the individual cells composing these organs and tissues revealed the fact that in the main the cytoplasm of the cells had suffered greater loss in size than the nucleus. There was distinctly observable an upset in the relative proportion of nuclear to cytoplasmic content of the cells with a resultant increase of the nuclear ratio. This was most conspicuous in the lining cells of the descending loop of Henle in the kidney, the secreting cells of the liver, cortical cells of the adrenal, and the cells of the chromaffin system. However, there was no alteration in the staining reactions of any of the cells or tissues.

A striking variation in the size of the nuclei of the liver cells was observable. The number of leucocytes and of red blood cells in the spleen was considerably reduced while the nodes were smaller than normal. The ovaries contained no corpora lutea and the ova were small. The follicular cells suffered, however, a marked reduction in number.

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[1] Randles and Knudson, *J. Biol. Chem.*, 1925, lxvi, 459.

[2] Gardner and Lander, *Proc. Royal Soc.*, London (B), 1913, lxxxvii, 229.

The most conspicuous and significant of the histologic changes were found, however, in the adrenal and testis. In the latter the cells lining the seminiferous tubules were remarkably reduced in number. The spermatozoa, spermatids, spermatocytes and column cells of Sertoli were absent, the cytologic lining of the tubules being reduced thus to a single layer of small cuboidal spermatogonia each possessing a relatively large centrally-placed nucleus. Tubules could be identified in which this lining stratum of spermatogonia cells was wanting in places. Occasionally, the lumen of the tubules was completely filled with exfoliated, deeply stained, atypical spherical spermatocytes of the first order and these likewise could be identified in the tubules of the epididymis. Most of the tubules were choked with a poorly stained undifferentiated mass of cellular detritus in which nuclei could not be made out. The interstitial cells were not reduced in number but were smaller than normal with a relatively large nucleus.

The zona reticularis of the adrenal was practically absent while the thickness of the zona fasciculata was considerably reduced. The thinness of the zona glomerulosa was referable chiefly to a reduction in the size of the large, clear, hexagonal cells of the intermediate layer.

The literature upon the subject of cholesterol synthesis presents many contradictory bits of evidence as to the importance of the rôle played by the various viscera in this function From the histologic evidence presented by this investigation one might fairly assume, and an interpretation might be made upon the basis, that most of the tissues and organs of the body are capable of a certain degree, at least, of this function In the adrenal the cortical cells and especially those of the zona reticularis possess this function but to a limited extent, whereas among the cells of the seminiferous tubules of the testis it is practically absent.

3989

Vitamin A in Relation to Growth and to Subsequent Susceptibility to Infection.*

H. C. SHERMAN AND M. P. BURTIS.

From the Department of Chemistry, Columbia University.

Previous investigations have repeatedly shown that graded responses in rate of growth may be obtained from the feeding of quantitatively graded allowances of either vitamin A or vitamin B, in each case as supplement to a basal diet adequate in all other respects. And in the case of vitamin B it has been shown, first by Osborne and Mendel[1] and later in a different way by Sherman and MacArthur,[2] that the larger the experimental animal the greater the amount of vitamin needed to maintain a given rate of growth, or the less the rate of growth resulting from the feeding of a fixed limited allowance of the vitamin. This is now found to be true of vitamin A also, larger experimental animals, of a given age and sex, growing more slowly upon a fixed allowance of this vitamin, the allowance in all of these cases being less than would have sufficed to support a fully normal rate of growth.

The bearing of this finding upon the accuracy of the quantitative determination of vitamin A by means of the feeding method is being discussed elsewhere. The purpose of the present communication is rather to invite consideration of the bearing of these and some related observations upon the nutritional rôle of vitamin A in relation to growth and health, with special reference to the importance of this factor in the food of children.

Thus the facts now demonstrated upon experimental animals (albino rats) that the vitamin A requirement increases both with size of body and with rate of growth will mean in the feeding of children that the large child needs a liberal allowance of this vitamin because of his size and the small child needs a liberal allowance to induce and support a more rapid rate of growth.

Our experiments have also shown that the level of intake of vitamin A (and perhaps certain other of the chemical factors in nutrition) during early life, may markedly influence subsequent susceptibility to infection. The characteristic susceptibility to infections which develops in animals suffering from lack of vitamin A is well known. We now find, further, that this influence of the level of intake upon the incidence of infection may be pronounced and long continued even when the differences of diet are no greater than may readily occur within the range of ordinary normal or adequate nutrition.

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* Published as Contribution No. 000, Department of Chemistry, Columbia University.

[1] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1922, liv, 739.

[2] Sherman, H. C., and MacArthur, E. H., *J. Biol. Chem.*, 1927, lxxiv, 107.

Young rats were taken, when 4 weeks of age, from families belonging to the same strain and which had lived under conditions identical in all respects except as to diet, 38 animals coming from Diet A (a mixture of one-sixth dried whole milk and five-sixths ground whole wheat) and 37 animals from Diet B composed of the same food materials but with a larger proportion of milk (one-third of the dry weight instead of one-sixth). All were alike placed upon vitamin-A-free food for periods of about 1 month, *i. e.,* until in each case the surplus store of this vitamin in the body was depleted, and then received during a further test period of 8 weeks the same limited allowance of vitamin-A-containing food. Autopsies at the end of this test period showed infections established in 75% of the animals which in early life had received Diet A and in only 25% of those which had during the same early period received Diet B with its larger proportion of milk. Taking account of the relative lengths of the rat and the human life cycle, and of the similarity of nutrition in the 2 species, this is an indication of the differences of incidence of infection to be expected among children of around 10 and 12 years, resulting from differences in the way they were fed before they were 3 years old.

In terms of the chief chemical factors involved, the better diet here used was richer not only in vitamin A but also in vitamin G, in calcium, and in certain of the nutritionally important amino acids. It is hoped that future papers from this laboratory may more fully differentiate the rôles of these several chemical factors.

The expenses of this investigation were shared by Columbia University and the New York Association for Improving the Condition of the Poor. We are greatly indebted to Mr. Bailey B. Burritt, General Director of the Association, for frequent cooperation, including grants from the Milbank Memorial Fund in aid of research of which the present paper records a part.

3990

Relation of the Antirachitic Factor to Reproduction in Birds.

A. F. HESS, W. C. RUSSELL, M. WEINSTOCK AND H. RIVKIN.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University, and the Department of Agricultural Biochemistry, Agricultural Experiment Station, New Brunswick, N. J.

Some years ago it was shown that hen's eggs are especially rich in the antirachitic factor, an observation which has been confirmed repeatedly. We have since found that the eggs of other animals, for example those of various fish and of serpents, likewise contain a large amount of this specific factor, the concentration varying considerably. In the hen the concentration is greater in the egg than in the liver. Several investigators[1] have shown that the content of the antirachitic factor in hen's eggs varies according to their diet and the degree of exposure to ultra-violet rays, and furthermore than when this factor is notably deficient a marked lack of hatchability results. This failure to hatch has been attributed to defective absorption of calcium from the shell into the yolk.

From last summer until this spring one group of hens has been placed on a diet devoid of the antirachitic factor (a grain mixture, including yellow corn, also meat scrap and 1 part of sodium chloride) but otherwise complete, whereas another group has been given daily supplements of cod liver oil. Our experience may be summarized by the statement that the group on the deficient diet laid comparatively few eggs even during the productive period of March and April, that these eggs contained very little of the antirachitic factor, that fertile eggs failed to hatch, and that the livers of these hens were also lacking in this substance. It should be added, however, that the blood serum of both groups of hens was found to contain a high percentage of calcium in the spring, that is during the productive season, in spite of the fact that one of the groups was laying and hatching normally, whereas the other was almost inactive in this respect.

The relation of the antirachitic factor to the development of the chick was studied from another aspect. Tests were made to ascertain whether this factor, which is so rich in the egg, is consumed in the course of the development of the embryo. It was found that the liver of the newly-born chick is practically devoid of this factor. Furthermore, a composite ethereal extract of the entire bodies of 50 newly-hatched chicks was found lacking in this factor when tested quantitatively on rats. These results would seem to give new significance to the function of what is commonly termed the antirachitic factor. The failure to hatch, associated with this nutritional deficiency, is a phenomenon due to the lack of development of the embryo. Complementary experiments, a report of which follows, were carried out on fish in connection with this study of reproduction.

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[1] Hart, E. B., Steenbock, H., Lepkovsky, S., and Kleitzien, S. W. F., *J. Biol. Chem.*, 1925, lxv, 579; Hughes, J. S., Payne, L. F., Titus, R. W., and Moore, J. M., *J. Biol. Chem.*, 1925, lxvi, 595; Bethke, R. M., Kennard, D. C., and Sassman, H. L., *J. Biol. Chem.*, 1927, lxxii, 595.

3991

Relation of the Antirachitic Factor to Reproduction in Fish.

A. F. HESS, C. E. BILLS, M. WEINSTOCK, E. HONEYWELL AND H. RIVKIN.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University, and the Research Laboratory, Mead Johnson Co., Evansville, Ind.

In a recent communication it was reported that in the cod there is a definite relationship between the percentage of calcium in the blood and the maturation period, the calcium rising to a markedly high level at the spawning season.[1] It was found likewise, in agreement with the investigations of others, that there is an inverse relationship between the size of the liver and the size and development of the roe, the liver becoming smaller as the ovary fills up and grows larger. At this time the liver may lose 50% or more of its fat. In many instances the weight of the fully matured ovary far exceeds that of the liver; for example in 3 fish, the ovary weighed 400, 345, and 270 gm. respectively, whereas the liver weighed 29, 105 and 60 gm. On testing the roe for antirachitic potency it proved in every instance to be active; this held true for the roe of the cod, sheepshead, flounder, carp, buffalo fish and smelt. The milt of the cod and of the carp had similar protective value. When the respective weights of the organs, of the liver and the ovary, were taken into consideration it was found that the latter contained quite as much as, or even more, of the antirachitic factor than the liver. In view of these facts it seems that the liver may be the source of the specific factor in the roe.

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[1] Hess, A. F., Bills, C. E., Weinstock, M., and Rivkin, H., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 349.

In the course of these experiments it was found that the antiracitic potency of the liver of the cod is in inverse ratio to its content of oil. Much to our surprise tests of the ether extract of livers, very small and poor in fat, showed them to be exceptionally potent —far exceeding in potency any oil which has been heretofore reported. The details of these tests will be published elsewhere.

Following the plan carried out in connection with an investigation of the antirachitic content of the egg of the hen and of the newly hatched chick, a biologic test was made of the fry. It was found that the fry of the cod, as well as of the smelt, was practically devoid of this factor which evidently had been used up in the course of the development of the larvae. Furthermore, the specific factor was not noted in trout 2 months old, although the liver of the adult fish was potent when as little as 0.1 gm. daily was fed.

It is believed that the antirachitic factor, or so-called vitamin D. is of essential importance in the development of the young fish as well as of the young chick. Whether this is to be interpreted as indicating a dual functional activity of this nutritional factor, or as due to the presence of an intimately associated factor, must be left for further study to determine.

3992

A Hemolytic Substance in Pneumococcus Culture Broth.

GRACE M. SICKLES. (Introduced by Augustus B. Wadsworth.)

From the Division of Laboratories and Research, New York State Department of Health, Albany.

In the course of previous studies,[1, 2] it was found that type I pneumococcus culture broth contained a substance which resembled the hemotoxin described by Cole[3, 4] and later by Avery and Neill.[5] Unlike the substance described by Cole, which was contained within the cells and was only set free by their dissolution, the hemolytic

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[1] Sickles, G. M., *J. Infect. Dis.*, 1927, xl, 369.

[2] Wadsworth, A. B., and Sickles, G. M., *J. Immunol.*, 1927, xiv, 321.

[3] Cole, R., *J. Exp. Med.*, 1912, xvi, 644.

[4] Cole, R., *J. Exp. Med.*, 1914, xx, 346.

[5] Avery, O. T., and Neill, J. M., *J. Exp. Med.*, 1924, xxxix, 745.

substance in the culture broth appeared, in some cases, as early as 4 hours after incubation was begun. The titer of the lytic substance, as indicated by the amount of culture broth required to produce an appreciable amount of hemolysis of 1 cc. of 1% washed sheep cells, was usually greatest after from 16 to 24 hours' incubation.

Culture broth of a group-IV pneumococcus strain, comparatively avirulent in its present form but capable of having its virulence raised by animal passage, and of a completely avirulent, atypical pneumococcus strain, No. 189, isolated from a horse which died during immunization with the standard type-I strain, No. 5, also contained the hemolytic substance.

The hemolytic substance in the culture broth, like that of Cole, is thermolabile and is destroyed by heating for 30 minutes at 55° C. Its activity is diminished by standing at cold room temperature and also, in a much shorter time, at 37° C.

Red cells, stroma, and leucocytes absorb the substance from the culture broth. The substance is also absorbed from the culture broth by shaking with animal charcoal and alumina. Shaking alone, however, greatly reduced the lytic power of the culture broth.

Normal horse serum has an inhibitory effect on the hemolytic power of the substance, while pneumococcus immune serum has a much more marked effect, which is apparently species rather than type specific.

3993

Comparative Study of Certain Aciduric Organisms of Intestinal and Dental Origin.

T. MORISHITA. (Introduced by L. F. Rettger.)

From the Laboratory of General Bacteriology, Yale University.

Therapeutic studies of certain intestinal disorders have increasingly directed attention toward a bacterial rôle played in the intestinal tract by naturally inhabiting types of organisms. This field has been advanced particularly through the work of Rettger and Cheplin[1] and the administration of *B. acidophilus* milk. These observations have in the main been supported.

Unfortunately this intestinal organism, *B. acidophilus,* has recently been confused with somewhat similar organisms which are associated with and may bear some relation to dental caries. The confusion has been increased not a little by the recent publications of Bunting and his co-workers[2] on a high acid-producing and aciduric organism in dental caries. They called this bacterium "*B. acidophilus*" without having apparently made any comparative studies of it and established strains of *B. acidophilus*. McIntosh and his co-workers,[3] and Rodriguez,[4] had previously reported the presence of an acid producing organism in dental caries which is seemingly identical with that of Bunting; they applied the name "*B. acidophilus odontolyticus*". More recently investigators have reported organisms associated with dental caries under the name of *B. acidophilus*, as Bunting had done.

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[1] Rettger, L. F., and Cheplin, H. A., *Arch. Int. Med.*, 1922, xxix, 357.

The determination of the relationship of the intestinal and the dental organisms appeared to the writer to be of extreme importance. This paper* presents a certain phase of the experimental work which has been conducted on more than 100 strains of acid-forming organisms isolated from dental caries, together with some known strains of *B. acidophilus* of intestinal origin. The results may be summarized briefly as follows:

(1) The occurrence of *B. acidophilus* of the intestinal type in dental caries is relatively rare compared with the other aciduric organisms found, and may be regarded as merely adventitious.

(2) A medium containing Klim-digest-casein and 1% glucose proved most satisfactory in this study.

(3) Morphologically the common aciduric tooth organisms appear as short, small rods occurring in chains, as compared with the larger and longer rods of *B. acidophilus* which under the given conditions tended to grow in long chains or threads.

(4) Colony formation of the 2 types is markedly different. The teeth organisms form solid, smooth, rather large colonies, while *B. acidophilus* has a tendency to produce the fuzzy or X type colonies, though at times the so-called Y type is quite apparent. The stroke plate method and the plating of the organisms in low dilutions will as a rule also show pronounced differences. Furthermore, the tooth organisms produce a general turbidity in broth culture, while with *B. acidophilus* the supernatant broth usually appears clear, along with distinct sedimentation in the bottom of the tube.

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[2] Bunting, R. W., and Palmell, *J. Am. Dent. Assn.*, 1925, xii, 381.

[3] McIntosh, J., James, W. W., and Lazarus, Barlow, *Brit. J. Exp. Path.*, 1922, iii, 138.

* Only 1 of the 5 groups, the one most frequently confused with *B. acidophilus*, is considered.

[4] Rodriguez, F. E., *Military Dent. J.*, 1922, v, 199.

(5) In sugar broth there is much greater acid production by the tooth organisms than by *B. acidophilus,* the former attaining a final pH of 3.8-3.0, and the other 4.4-3.8.

(6) In the determination of limiting pH for the growth of the 2 types, the tooth organisms grew in a medium having a far greater range of pH (both acid and alkaline), than did *B. acidophilus.*

(7) In the study of the reduction of methylene blue in milk, except in a few instances, the tooth organisms reduced the indicator before coagulation of milk took place. On the other hand *B. acidophilus* first produced a milk coagulation and then brought about reduction gradually in the depth of the tubes.

(8) The tooth organisms were able to grow at temperatures as low as 12-14° C., while *B. acidophilus* failed to do so even at 18° C. This suggests, perhaps, one of the most important means of separating these 2 types.

(9) The ability or inability of the 2 types of organisms to ferment certain sugars, to coagulate milk, and to grow at low temperatures was studied. Twenty-two different sugars were used.

Both of the representative tooth strains belonging to this group coagulated milk, and produced acid from mannose, trehalose, melezitose, mannit, sorbitol and salicin. They did not attack raffinose. Sucrose was fermented by some strains, but by relatively few of the non-raffinose fermenters, compared with other groups of tooth organisms. On the other hand, *B. acidophilus* fermented raffinose and sucrose, but did not attack mannite and sorbitol.

(10) Agglutination experiments also showed differences. There was no cross agglutination between the 2 groups in dilutions of immune serum higher than 1:100, while both types were agglutinated by their homologous sera.

Conclusion: The present comparative study seems to the writer to warrant the conclusion that the tooth organisms discussed here constitute a type entirely separate from *B. acidophilus* of the intestinal tract.

3994

Relation of Vitamin "B" to Infection and Immunity with Special Reference to Bacillus Welchii.

W. B. ROSE. (Introduced by George R. Cowgill.)

From the Laboratories of Physiological Chemistry and Bacteriology, Yale University, and the Graduate School of Medicine, University of Pennsylvania.

During the course of an investigation in which dogs were fed a diet deficient with respect to vitamin "B" one animal developed severe convulsions. Death seemed imminent. The only chance to save its life was the immediate parenteral injection of a vitamin "B" concentrate (Cowgill[1]). Vitamin powder (Harris) was dissolved, neutralized, sterilized by boiling for 3 minutes and the solution injected subcutaneously. Practically all symptoms were alleviated within a few hours.

A few days later an abscess developed at the site of the injection. Palpation revealed gas crepitus. It was then recalled that the material injected had not been sterilized sufficiently to destroy resistant spores. A provisional diagnosis of "gas infection" was therefore made. The abscess was incised and drained and a pure culture of *Bacillus aerogenes capsulatus* isolated. The animal was given large doses of material rich in vitamin "B" daily and recovered rapidly. Repeated cultures subsequently made from the site of the wound proved to be negative for *B. welchii.*

The following questions arose: (1) are normal dogs immune to this organism? (2) if normal dogs possess "natural" immunity, is it possible for a lack of a dietary essential to bring about a breakdown of this immunity? (3) was the vitamin "B" therapy responsible for the very rapid development of immunity? An attempt was made to answer these questions by experiment.

Dogs, presumably normal, were inoculated with varying quantities of cultures of this organism. No untoward symptoms were observed. The animals were then fed a diet complete in every known respect except for what has hitherto been called vitamin B. *Positive blood cultures were obtained.* Vitamin "B" therapy was then instituted and the blood cultures became negative. This procedure was repeated a number of times over a period of 15 months and the same results obtained.

The possibility that the lowered resistance exhibited by these vitamin "B" deficient dogs resulted from a chronic state of inanition incident to starvation was eliminated by the following procedure. The animals were fed a diet containing a minimum supply of vitamin "B". This, however, was adequate to maintain a perfect appetite and resulted in a gain in body weight. Nevertheless, frequent positive blood cultures were obtained. The dogs were then given large doses of vitamin "B"—containing material and the blood cultures remained negative for months.

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[1] Cowgill, G. R., *Am. J. Physiol.*, 1923, lxvi, 164.

As a result of other experiments in collaboration with Dr. C. J. Stucky, which will be reported elsewhere, the development of anhydraemia in "B" avitaminosis was observed. An attempt was made in these immunologic studies to see whether or not anhydraemia was a contributing factor resulting in an increased susceptibility to infection. These studies are in progress at present and will be reported on in the near future.

In general the results appear to indicate that nutritional factors deserve important consideration in immunologic studies, and that vitamin "B" in particular merits special attention.

3995

Respiratory Metabolism of Excised Normal and Diabetic Tissue.

HENRY B. RICHARDSON, ROBERT O. LOEBEL, AND EPHRAIM SHORR.

From the Russell Sage Institute of Pathology, New York City.

Studies were made of excised kidney and muscle tissue of normal and depancreatized dogs, in order to answer the following questions:

(1) With normal animals what is the respiratory metabolism of these tissues when removed from the body?

(2) In excised diabetic tissue (a) is the power to oxidize carbohydrate lost? (b) is the power to split carbohydrate into lactic acid lost?

The main features of the technique were (1) the employment of the Barcroft-Warburg manometers in the study of micro-respiration, (2) the comparison of tissues of normal and diabetic animals, (3) parallel determinations of the metabolism of the tissues of the same animal, and (4) the development of a technique for obtaining relatively untraumatized mammalian muscle, by stripping the fasciculi lengthwise, instead of mincing.

The results are as follows:

(1) The figures for oxygen consumption of kidney and muscle

were remarkably constant. For instance in normal muscle the extreme variations in 24 consecutive determinations were from 2.3 to 3.1 cu. mm. per dry mg. per hour. These correspond to values obtained in both tissues by the method of analyzing arterial and venous blood simultaneously in the intact organ. The respiratory quotients were all within physiological limits and indicated that various mixtures of foodstuffs were oxidized. In 11 triplicate determinations the highest figure was 0.911. This cannot be reconciled with the widespread idea that only carbohydrate can be oxidized by muscle.

(2a) The average respiratory quotients were as follows:

Kidney—Normal without foodstuff	0.812
With glucose	0.873
Depancreatized, without foodstuff	0.694
With glucose	0.684
With lactate	0.734
Muscle—Normal with glucose	0.874
Depancreatized, with glucose	0.728

These figures show that isolated diabetic tissue can oxidize only a small amount of carbohydrate.

(2b) Diabetic tissue retains the power of splitting carbohydrate into lactic acid. Therefore it is not a failure to glycolize which depresses the oxidation of carbohydrate in diabetes.

3996

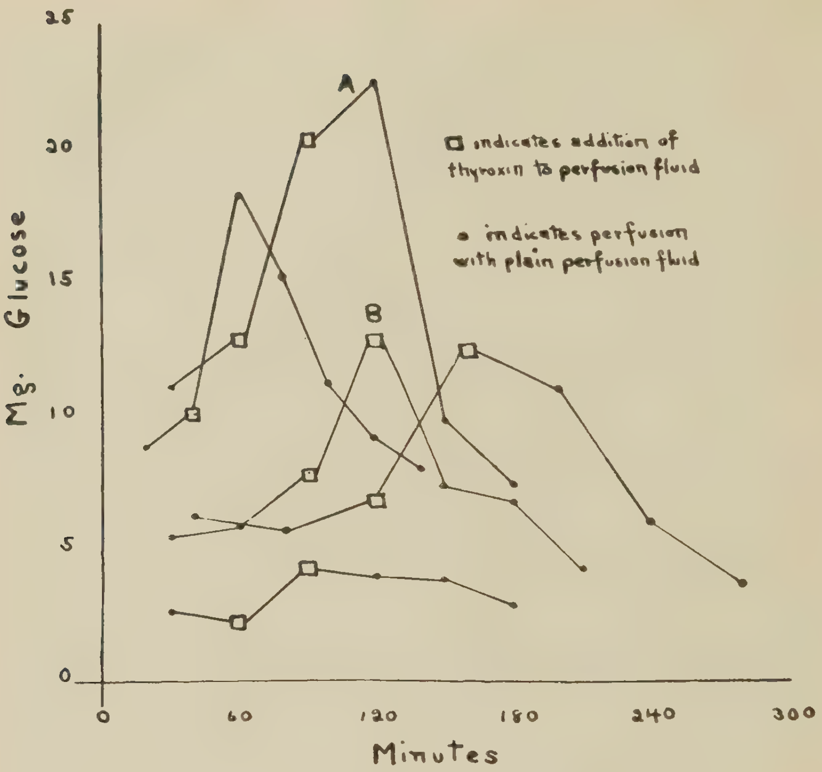
Effect of Thyroxin on Formation of Sugar in Surviving Liver of Winter Frog.

NATHAN F. BLAU AND HELEN McNAMARA.

From the Laboratory of Experimental Biochemistry, Johnson Livingston Fund, Cornell University Medical College.

This study was made upon the perfused liver of the bullfrog *(Rana catesbiana)*. It was found that in the isolated livers, whether excised or left *in situ*, thyroxin as a rule produced a slight fall in the amount of sugar given off into the perfusate, as against the amounts of sugar obtained during the preliminary control periods.

When the body of the frog was perfused with thyroxin through the systemic aorta there was a marked rise in the sugar of the perfusate draining from the hepatic vein. (Graph 1.) This increase lasted only a limited length of time and was then followed by a grad-



GRAPH 1.

Effect of thyroxin on the hepatic sugar of the perfused water frog. Dose of thyroxin approximately 1 mg., dilution 1:20,000-1:100,000. A represents perfusion of frog with renal vessels ligated. B represents perfusion of same frog after removal of the ligatures.

ual decline of the sugar concentration to the control level. The fall occurred in spite of prolonged perfusion with thyroxin. The same results were observed in a frog with the adrenals excluded from the circulation.

When the liver was tied off so that it could be perfused separately from the body, and the body perfusates, collected from the posterior vena cava, were passed through the isolated organ, there was a sharp and prolonged increase in the hepatic sugar. There was no further rise, however, as a result of perfusion with body perfusate which had thyroxin added to it before passing it through the body. In an experiment with 2 frogs which were cross-perfused, the fluid from the vena cava of the one frog emptying through a rubber tube connection into the liver of the other, there was a 22% rise in the hepatic sugar. At the end of the experiment this liver was found to contain only 6.10 mg. of glycogen.

These results seem to point to the conclusion that the effect of thyroxin upon the carbohydrate of the liver is probably an indirect one. The manner in which this influence is brought about is not yet clear.

3997

Respiratory Anaphylaxis in Guinea Pig Due to Castor Bean Dust.*

BRET RATNER AND HELEN LEE GRUEHL.

From the Departments of Immunology and Pediatrics, University and Bellevue Hospital Medical College, New York University.

Last year we[1] demonstrated for the first time that normal guinea pigs, when exposed to an organic dust such as horse dander, could become sensitized through inhalation. Guinea pigs, thus sensitized, when again placed in contact with the same dust after a suitable incubation period, demonstrated unmistakable signs of anaphylaxis which we termed "respiratory anaphylaxis" and which we believed to be identical with bronchial asthma in the human being. This was offered as a method for studying asthma experimentally in the animal.

Recently, Figley and Elrod[2] reported the occurrence of a large number of cases of asthma resulting from the inhalation of castor bean dust "pomace" thrown into the air from the pipes of a castor oil factory. To determine whether pomace was an anaphylactogen and could be the cause of asthma, we proceeded to study the problem of pomace asthma in the guinea pig. The pomace came from the above castor oil factory.

Thirty-seven normal guinea pigs were placed for several hours in glass cages in which pomace was kept in circulation by currents of air. After an incubation period of about 3 weeks, 2 of these animals were given an intravenous injection of 0.5 cc. of a crystal clear alkaline extract of this pomace. Both animals showed definite anaphylaxis, one dying within a few minutes and demonstrating

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* This work is being carried on under "The Crane Research Fund for the Study of Allergic Diseases in Children."

[1] Ratner, B., Jackson, H. C., and Gruehl, H. L., *Am. J. Dis. Child.*, 1927, xxxiv, 23.

[2] Figley, Karl D., and Elrod, Robert H., *J. Am. Med. Assn.*, 1928, xc, 79.

typical anaphylactic lungs, and the other going into collapse with marked dyspnea and finally recovering. These 2 animals established the fact that sensitization could be accomplished by inhalation of pomace.

Of the remaining 35 animals, 17 died 6 to 7 days after the sensitizing inhalation of pomace, with definite evidences of ricin poisoning. However, besides the classical abdominal pathology of ricin poisoning, we noted in many of the animals a severe hemorrhagic condition of the lungs. We first attributed this lung condition to the entrance of ricin through the nasal passages but we obtained similar results later when the pomace extract was injected.

After an incubation period of 2 to 3 weeks, the remaining 18 animals were again placed in the cage to determine whether we could demonstrate the symptoms of respiratory anaphylaxis (bronchial asthma). Unmistakable signs of respiratory anaphylaxis were shown by these animals. Later, however, 10 of the 18 died from ricin poisoning with the pathological findings noted above. The remaining 8 were injected intravenously with pomace extract and showed typical symptoms of anaphylaxis.

In testing the toxicity of the pomace extract used, we readily discovered that the normal animal died from typical ricin poisoning in about 12 hours after an intravenous injection of the extract. It was interesting, however, to note that the primary toxicity was never manifested immediately after injection and never in the same manner in which anaphylaxis was manifested. Further control tests with ricin gave results identical with those obtained with pomace.

From the 8 cases noted above which showed typical anaphylaxis on injection of pomace extract, a definite immunity to ricin poisoning was demonstrated. In 3 of these animals there was no evidence of ricin poisoning even after prolonged contact with the pomace.

3998

Effects of Experimental Block of the Amphibian Nervous System.

J. S. NICHOLAS.

From the Osborn Zoological Laboratory, Yale University.

Experiments previously reported[1] have shown that the cut ends of the spinal cord can be prevented from reuniting by the removal of a comparatively large amount of the nervous system and the implantation into the wound of the anlage of the limb and pronephros.

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[1] Nicholas, J. S., *Anat. Rec.*, 1928, xxxviii.

The responses of such an embryo during early stages are strictly in keeping with the findings of Coghill and Harrick. The uninjured portions of the nervous system behave as individual units and take up their function with a very slight delay over the normal. As the animals differentiate, a complex series of reactions occurs by means of which stimuli are transmitted across the gap in the nervous mechanism. This transfer of stimuli takes place through the intermediation of the musculature which is in that region.

The first integration of response was noticed in the reactions occurring after stimulation in the head region. The sensory pick-up is mediated through the uninjured medulla giving rise to a motor response through the 7th cranial nerve which activates the gular musculature and the arcuate muscles of the gills. This reaction is transmitted to the anterior trunk region where the sensory pick-up acts in the pectoral region to transmit the stimulus to the uninjured cord of the trunk. This motivates the musculature of the flank and tail and gives rise to distinct swimming movements.

The limbs which have been transplanted into the wound area develop synchronously with the normal limbs. They are generally single and although they develop in close proximity to the gills, show no trace of the developmental dominance of that tissue. In every case the laterality of these limbs has been that of the side from which they were taken.

The innervation of the transplanted limbs is through the 7th nerve. They possess to some degree all the primary movements found in the limb in this form. For a long period after the operation, the transplanted limb moves only when there is movement of the gills, but in later stages the limb is capable of flexion and extension of the whole limb or any of its parts without perceptible motion in the gills.

Other authors have stated the conditions which may limit the movement of transplanted limbs,[2] and have shown that function may be limited by the morphological deficiencies of the transplanted parts as well as due to nervous deficiencies. The present experiments show that a transplanted limb even with a very abnormal nervous supply is capable of having all the primary movements. The amount of movement, however, is quantitatively incomplete.

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[2] Detwiler, S. R., *J. Comp. Neur.*, 1925, xxxviii.

3999

Effect of Contact with Tissues In-Vitro on Activity of a Chicken Tumor Agent.

F. DURAN REYNALS* AND JAMES B. MURPHY.

From the Laboratories of the Rockefeller Institute for Medical Research.

The filtrate of Chicken Tumor No. 1 when kept in contact for 1 to 5 hours at room temperature with finely minced or dried muscle from susceptible fowl is generally either completely inactivated or has its potency greatly reduced. This partial or total inactivation was pronounced in 34 out of 46 tests. Carefully controlled experiments have shown that the 12 cases in which there was no demonstrable reduction in potency of the filtrate after muscle contact was explained by the fact that excessively active filtrates were used, for the inactivating effect of the muscle became evident when such filtrates were tested in more dilute form.

The specific nature of this reaction was shown by the fact that not only did muscle tissue of such resistant animals as the rabbit and pigeon have no effect on the activity of the tumor filtrate but the brain, kidney and liver of the susceptible chicken were without activity in this respect.

Desiccation of chicken muscle pulp after contact with filtrates has failed to release the tumor agent in an active form from the muscle. Either the union of the agent with the muscle substances is so strong as to render dissociation difficult or the phenomenon may be one of inactivation.

4000

The Availability of Nitrogen Compounds for the Streptococcus.

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This report deals with the effect upon growth and viability of streptococci, of the addition of various nitrogen compounds to synthetic media. Details of the technique involved have previously been described.[1, 2] The substances tested include inorganic nitrogen compounds, amino nitrogen compounds, benzene and imidazo derivatives and alkaloids.

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We found that $(NH_4)_2HPO_4$ is more available for streptococcus than $(NH_4)_2CO_3$, NH_4NO_3 and $(NH_4)_2SO_4$. Previous investigators make no mention of such findings as applied to streptococcus metabolism. However, Dolt,[3] in his study of about 40 simple media for the growth of *B. coli* has chosen 2 as the best. One of these contains $(NH_4)_2HPO_4$ as the sole source of N. Fermi[4] shows that this ammonium salt is easily utilized by *B. prodigiosus* and *B. fitzianus.*

The relation of amino acids to the growth of streptococcus is not constant. For example, one series of experiments, in which amino acids were added to a basal mixture, showed glycocoll more beneficial than aspartic acid; in another series of experiments, the same amino acids were added to another basal mixture, aspartic acid was in the lead and glycocoll fell behind. Similar variable effects were obtained between aspartic acid and alanine and glycocoll and alanine. Tyrosine was always less available than glycocoll in these studies. The relation between tyrosine and aspartic acid was not so regular. It is evident that no definite conclusion can be drawn as to the comparative utilization of a certain group of compounds when a single basal mixture is investigated.

When amino acids were compared with other N compounds 2 tendencies were noticed. In some cases the former were utilized less rapidly than the latter. There were, however, comparatively few instances in which this was true. Thus, NH_4 lactate>leucine and tyrosine; uric acid>glycocoll>aspartic acid, glutamic acid and tyrosine, and NH_4 acetate $=$ $(NH_4)_3$ citrate>aspartic acid. These several comparisons considered en masse simply mean that NH_4 lactate, uric acid, NH_4 acetate and $(NH_4)_3$ citrate supported the viability of the organism longer than the amino acids. It may be well to draw attention to the finding: Albumen>histidine>tryptophane>leucine and uric acid>glycocoll>aspartic acid, glutamic acid and tyrosine. Although albumen *per se* is not classed as an amino acid, amino acids are easily liberated from it. The writers feel that the result is due to the presence of some derived albumen substances in the autoclaved medium. Undoubtedly, amino acids were among

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[1] Krasnow, F., Rivkin, H., and Rosenberg, M. L., PROC. SOC. EXP. BIOL. AND MED., 1925, xxiii, 215.

[2] Krasnow, F., Rivkin, H., and Rosenberg, M. L., *J. Bact.*, 1926, xii, 385.

[3] Dolt, M. W., *J. Infect. Dis.*, 1908, v, 616.

[4] Fermi, C., *Archiv. für Hygiene,* 1892, xiv, 1.

them. So that for the purpose at hand, albumen may be classed among the amino acids. There were then altogether 3 instances in which the 4 "non-amino acid" substances were better than amino acids. On the other hand most of the latter were better than most of the former. This was well illustrated. Alanine, glycocoll, leucine, tyrosine>NH_4 acetate, $(NH_4)_2CO_3$, $(NH_4)_3$ citrate, NH_4 lactate, NH_4NO_3, $(NH_4)_2$ oxalate, $(NH_4)_2HPO_4$, $(NH_4)_2$ tartrate, caffeine and urea; glycocoll, alanine, leucine, aspartic acid>NH_4 acetate, betaine and urea; histidine, tryptophane, leucine and glycocoll>NH_4 lactate and urea. The contrast is clear. In general, amino acids supply the streptococcus with N needs better than do other N compounds.

In this study, too, as was the case with carbon compounds,[5] there were instances of N addition causing no effect on the growth of the organism. This was true of $(NH_4)_2CO_3$, $(NH_4)_2HPO_4$, urea, aspartic acid, glutamic acid, tyrosine, uric acid and NH_4 lactate.

Tests on a series of 70 media containing N in imidazo, pyrole, pyridine, quinoline, azo, nitro-benzene and alkaloid linkages showed that streptococci can utilize the N from anthranilic acid, indigo and neosalvarsan.

4001

Studies on Anaphylaxis with the Products of Peptic Digestion of Proteins. II.

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From the Laboratories of The Rockefeller Institute for Medical Research.

In the experiments reported previously[1] material was used for the peptic digestion consisting mainly of egg albumin but also containing egg globulin. Since it was thought that possibly the latter was responsible for the effects observed, experiments were made with preparations of egg globulin. The results were in agreement with the former experiments in that the animals sensitized with the peptic digestion products were more sensitive to these than to unchanged globulin and vice versa. This result was especially pronounced in one batch of guinea pigs, the lethal intravenous dose for the animals sensitized with the digestion product being 1 cc.

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[5] Krasnow, F., and Rosenberg, M. L., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 295.

[1] Landsteiner, K., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 540.

of a 1-2% solution of the digested material* and about 1 cc. of a 0.2% solution of globulin, while the respective values for the animals sensitized with globulin were about 4% and 0.0125%. In another batch of animals this difference was still definite but less considerable. But, after precipitating a 5% solution of the digestion product with an equal volume of 10% trichloracetic acid and precipitating the supernatant fluid with alcohol the resulting substance was much more toxic for the guinea pigs sensitized with the digested material (lethal dose 0.5%) than for those sensitized with globulin (lethal dose 4% or more). Similar results were obtained by sensitizing with digested heat-coagulated egg globulin. These animals were killed by an *i. v.* injection of 1 cc. of 0.25% to 0.125% of the digestion product after fractionation with trichloracetic acid and by 1 cc. of a 0.1% solution of unchanged globulin.

In some cases, but irregularly, the animals sensitized with peptic products succumbed to injections of 1% ovomucoid. This substance, however, can hardly be essential for the results reported since digested products of heat-coagulated globulin were more active in producing shock than those of non-coagulated globulin in animals sensitized with digestion products of the latter preparation, and also on account of the results of experiments with guinea pigs sensitized with ovomucoid itself.

4002

Estimation of the Acid Combining Capacity of a Protein by Means of the Interferometer.

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From the Department of Chemistry, Columbia University.

The index of refraction of a mixed solution of gelatin and of hydrochloric acid as measured by an immersion refractometer has been stated to be an additive function of the indices of refraction of the individual solutions.[1] Using the Zeiss-Löwe interferometer, which is a differential refractometer capable of measuring small changes in refractive index to which an immersion refractometer is insensitive, the authors have found that the refractive indices of mixed solutions of gelatin and of hydrochloric acid are not additive.

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* The lethal dose of a digestion product obtained from heat-coagulated egg globulin was 1 cc. of a 0.3 to 0.2% solution.

[1] Walpole, G. S., *Kolloid Z.*, 1913, xiii, 241.

TABLE I.

(0.881 gm.* of ash-free gelatin and varying proportions of 0.1004 Normal Hydrochloric Acid in 200 cc. total volume.)

cc. of acid added	Interferometer reading (Average of 5 determinations)
0	1870
1	1875
2	1881
3	1886
4	1891
6	1902
8	1914
10	1934
15	1985
20	2036

* Absolute, water-free basis.

However, the refraction of mixed solutions of gelatin chloride and of hydrochloric acid are additive. Consequently, when a solution of gelatin is titrated with an acid, an abrupt change in slope of the curve (refraction as function of acid added) is noted at the

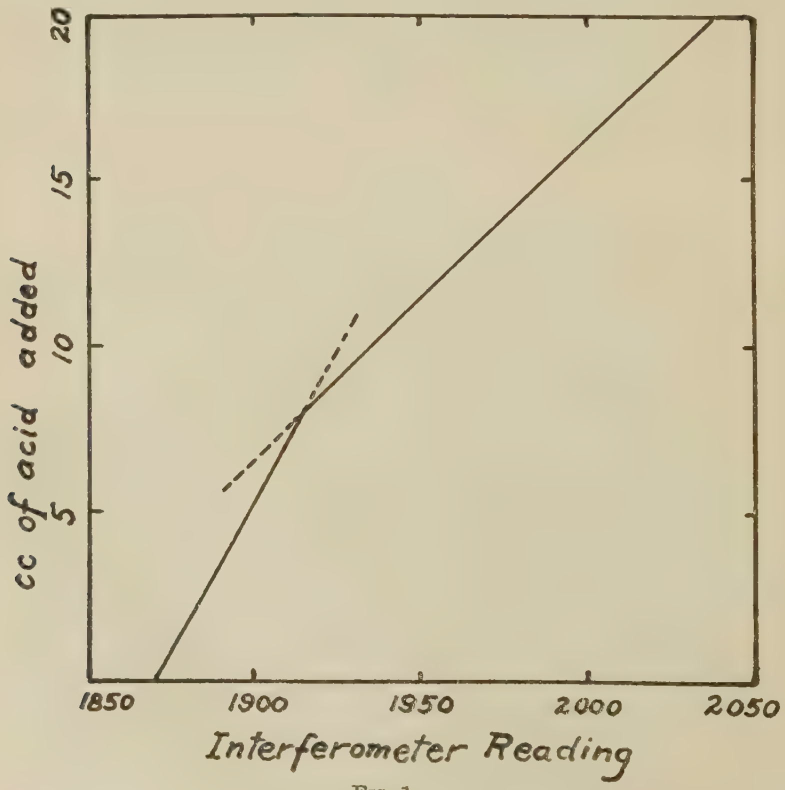

FIG. 1.

stoichiometric point. The authors have found this to be the case also when an amino acid is similarly titrated, and recently the interferometer method of following simple acid-base neutralizations has been reported elsewhere.[2]

The data for a gelatin titration are given in Table I and are shown graphically in Figure I. The gelatin used was a pure specimen obtained from the Eastman Kodak Company. Its ash content was 0.03%.

The 2 branches of the curve intersect at the point equal to about 7.9 cc. of acid. Consequently the equivalent weight of this specimen of gelatin is about 1111. For the benefit of the reader interested in a description of the instrument used by the authors, the excellent paper of Adams[3] is recommended.

4003

Rickets in Rats. VI.* Effect of Phosphate Added to the Diet of Non-Ricketic Rats.

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Phosphate added to the diet causes rapid healing of rats made ricketic on a high calcium-low phosphorus regimen which has not been irradiated and is poor in vitamin D. This is evidenced by histological sections of the bones, blood serum analyses, bone analyses and studies of the metabolism of calcium and phosphorus.[1] As a result of the alteration in diet not only is the rickets cured but a transient tetany is also produced. The blood serum phosphate becomes extremely high—the calcium low. If the animals survive the tetany the blood serum calcium and phosphorus return to values considered normal in 2 weeks. Similarly, the feeding of phosphate to normal dogs is known to produce tetany (Salvesen, Hastings and McIntosh[2]).

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[2] Berl, E., and Ranis, L., *Berichte* (B), 1928, lxi, 92.

[3] Adams, L. H., *J. Am. Chem. Soc.*, 1915, xxxvii, 1181.

* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

[1] Karelitz, S., and Shohl, A. T., *J. Biol. Chem.*, 1927, lxxiii, 665.

[2] Salvesen, H. A., Hastings, A. B., and McIntosh, J. F., *J. Biol. Chem.*, 1924, lx, 311.

When the diet for rats was altered by the addition of phosphate it was not considered a high phosphorus diet. It was known from the studies of McCollum *et al.*[3] to produce normal rats. The question accordingly arises whether non-ricketic animals shifted from a diet of low phosphorus to one of moderate phosphorus content would develop tetany. If so, would this be of short duration like that observed in the cure of rickets? Or is there something in the condition called rickets which predisposes to an exaggerated effect of phosphate?

Plan—Rats fed with diets of a high calcium low phosphorus content and a liberal supply of anti-ricketic agents were compared to those fed on the same diet without vitamins. After 3 weeks on this diet all were given enough phosphate to make the Ca/P ratio 1/1. Seven and 14 days later the blood serum was analyzed for calcium and inorganic phosphorus, histological preparations of the bones were made, and the percentage ash in the fat-free bones was determined.

Procedure—The basal diet, which produces marked rickets, was Steenbock and Black's diet No. 2965. It consists of 76% of yellow corn, 20% of wheat gluten, 1% of NaCl and 3% of $CaCO_3$. 10% of lard was added. This diet was fed to the group which served as a rickets control. The ratio of Ca/P was 4.25. Diet I was prepared from fine ground yellow corn which had been exposed to ultraviolet rays from a Hanovia lamp for 20 minutes at a distance of 24 inches. To 100 gm. of diet were added 8 gm. of lard and 2 gm. of cod liver oil, which had been previously tested and was rich in vitamins A and D. The ratio of Ca/P, as in the original diet, was 4.25. Diet II was the same as Diet I, except that enough sodium acid phosphate was added to make the ratio of Ca/P 2.0. Diet III was the same as Diet II, except that the corn was not irradiated.

The rats were fed on Sherman's normal diet B during pregnancy and lactation. The young were separated from their mother at 21 days and continued on Sherman's normal diet for 1 week only when they were given the experimental diets. Each group of animals was fed one of these experimental diets for 21 days. All were then changed to a diet similar in all respects to that which they had previously received but with the addition of enough sodium acid phosphate in every case to make the phosphorus content equal to that of the calcium. After 7 and 14 days on the altered diet, they were anesthetized lightly by ether and killed by bleeding. The blood serum of 3 or 4 rats was pooled and analyzed for calcium and inorganic phosphate. The bones were freed from tissue, dried, extracted with alcohol and ether and the ash determined. The methods have been described previously.[1]

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[3] McCollum, E. V., Simmonds, N., Shipley, P. G., and Park, E. A., *Bull. Johns Hopkins Hosp.*, 1922, xxxiii, 296.

TABLE I.
Effect of Moderate Phosphate Additions to the Diet of Ricketic and Non-ricketic Rats.

Diet				Serum		Bones
	Vitamin added	Ca/P	Days on added P	Ca	P	Ash of fat-free bones
				mg. %	mg. %	%
Sherman B Normal control	None	0.85	0	10.6	9.8	57.4
I	C.L.O.+ irrad.	4.25	7	10.6	9.0	53.0
			14	10.9	10.4	58.0
II	C.L.O.+ irrad.	2.0	7	10.9	9.0	58.0
			14	10.9	9.3	58.4
III	C.L.O.	2.0	7	10.6	9.8	57.6
			14	10.3	10.2	58.0
Basal	None	4.25	7	7.0	13.0	—
			14	8.2	7.6	46.2
Basal Rickets control	None	4.25	0	12.1	1.5	33.5

The high calcium-low phosphorus diets were fed for 21 days and then altered by the addition of sufficient NaH_2PO_4 to make the calcium and phosphorus equal in amount.

Results: The values for the serum calcium and phosphorus and for bone ash are given in Table I. As a standard of comparison values obtained from animals fed Sherman's normal diet are included. The rickets control group showed low serum phosphorus and low ash values characteristic of rickets produced with this diet. The ricketic animals which received added phosphate had serum which was high in phosphate and low in calcium. The bones increased considerably in ash content. The blood serum analyses for the animals on Diets I, II and III show neither a rise in phosphorus nor a drop in calcium and are essentially normal. The histological preparations showed normal bones. The ash content of the bones is normal also.

Ricketic rats develop tetany and have high phosphorus and low calcium in the blood serum when moderate amounts of phosphate are added to the diet. Rats fed with the same diet, plus anti-ricketic vitamins, show neither tetany nor an alteration in blood calcium and phosphorus and their bones yield a normal amount of ash.

4004

On the Development of Isoagglutinins Following Transfusions.

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Observations on individual differences in human blood aside from the 4 blood groups[1, 2] support the idea that in transfusion the blood of the donor and recipient is, as a rule, not identical in biochemical constitution even though belonging to the same blood group.

In the literature instances are reported in which the first transfusion was uneventful while a repeated transfusion with the same compatible donor was followed by severe symptoms,[3, 4, 5, 6, 7] and others. There are, however, only casual remarks on the occurrence of atypical isoantibodies under such conditions.

We examined recently the sera of 4 individuals of group 0 and 3 of group A.* In 5 of these individuals, who had been transfused several times with the blood of the same donor of their own group, no abnormal agglutinins were observed. The 6th individual, of group 0, was transfused with blood (500 cc.) of the same group and the transfusion was repeated, using the same donor, since in the routine test no incompatibility was detected. Actually no untoward manifestation followed. On careful observation with a more delicate technique, however, an abnormal isoagglutinin was found in the serum of the transfused individual (after the 1st and 2nd transfusion) that reacted on numerous bloods and stronger on several others than on that of the donor. The reactions took place with bloods of group 0 and, as could be shown after removal by absorption of the common isoagglutinins, also with bloods of the other groups. Some of the results are recorded in the table. The

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[1] Landsteiner, K., and Levine, Philip, PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 600, 941.

[2] Landsteiner, K., and Levine, Philip, *J. Exp. Med.*, 1928, xlvii, 757.

[3] Lindemann, E. E., *J. Am. Med. Assn.*, 1914, lxii, 993.

[4] Sydenstricker, V. P. W., Mason, V. R., and Rivers, T. M., *J. Am. Med. Assn.*, 1917, lxviii, 1677.

[5] Libman, E., and Ottenberg, R., *Trans. College Phys.*, 1917, xxxix, 266.

[6] Waugh, W. G., *Brit. Med. J.*, 1919, ii, 39.

[7] Bowcock, H. M., *Bull. Johns Hopkins Hospital*, 1921, xxxii, 83.

* The terminology of the 4 blood groups used in this publication is that recommended by the American Association of Immunologists and by a committee of the National Research Council.[8]

[8] Editorial, *J. Am. Med. Assn.*, 1927, lxxxviii, 1421.

tests were made by adding in small test tubes 1 drop each of saline, serum, and 2.5% suspension of washed blood cells. After the mixture stood for 2 hours at room temperature readings were made by withdrawing a drop on a glass slide for microscopic examination. The reactions became distinct after about 30 minutes.

At the end of 2 hours the strongest reactions were similar to common isoagglutination of moderate strength. The reactions persisted at 37° with but little diminution. By adding a sufficient amount of sensitive blood the agglutinin proved to be absorbable.

TABLE I.
Tests with post transfusion serum No. 191.

	Group 0								Group B			
No. of blood specimen	191	195	231	235	248	255	257	261	207	208	1122	1226
Reaction	0	+	0	0	+±	0	+	0	±	0	+	0
	Group A								Group AB			
No. of blood specimen			213	233	520	726	851	1179	614	621	861	1206
Reaction			+	+	0	+	0	0	+±	0	0	0

The blood of the donor was No. 195.

The incidence of the reactions was as follows: Group 0, 20+ reactions, 40— reactions; Group A, 16+, 12—; Group B, 6+, 9—; Group AB, 3+, 6—. With regard to the occurrence in all groups, the reactions recall those obtained with immune agglutinins[1, 2] and the abnormal isoagglutinin recently described by Ottenberg and Johnson[9] (*cf.* 2). When tests were made with numerous individual bloods no parallelism was observed between the effects of the immune sera mentioned and the transfusion serum. On comparing the latter with the serum described by Ottenberg and Johnson corresponding reactions were found in a number of specimens but distinct discrepancies in others.

After observing the abnormal reactions reported, we repeated the test with the serum of the recipient drawn prior to the first transfusion, and the cells of the donor. The results were again negative. On making the tests with the same serum and other bloods a trace of agglutination was noticed with 2 bloods which gave the strongest reactions with a post-transfusion serum. Hence one may conclude that an agglutinin of very slight activity was present originally in the serum of the recipient and that the amount of this agglutinin was increased as a result of the transfusion.

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[9] Ottenberg, R., and Johnson, A., *J. Immunol.*, 1926, xii, 35.

In the serum of another transfused individual (group A)† we found agglutinins which acted weakly on all bloods 0 and AA_2, only faintly on some AA_1‡ thus behaving in a manner similar to the cold agglutinin α^2 described previously.[10] The reactions disappeared at 37°.

On account of the observations reported it will be of interest to make a systematic study of post-transfusion sera in order to establish how frequently atypical agglutinins may occur.

By means of the immune agglutinins referred to above we were able to apply the principle used by Ashby[11] for the demonstration of donor's blood in the circulation of the recipient, also for cases where donor and recipient belong to the same blood group. In this way the foreign blood could be found for as long as 7 weeks after the transfusion. Investigations after a longer interval have not yet been made.

In connection with the reaction of the pretransfusion serum it may be stated that, on examining at room temperature (about 20°) numerous sera, some were found which gave atypical isoagglutination reactions (*Cf.* Unger,[12] Guthrie and Pessel,[13] Jones and Glynn,[14] Landsteiner and Witt.[15]) One of these sera, of group AB agglutinated at room temperature blood of group 0 and AA_2 and to a lesser degree some bloods of group B. The reactions with this serum disappeared at 37°. It is intended to describe these observations in detail.

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† For this specimen we are indebted to Dr. M. Lederer of the Brooklyn Jewish Hospital, who noticed some irregularity in grouping this blood. The pretransfusion specimen was not tested by us.

‡ For the terminology see [10].

[10] Landsteiner, K., and Levine, Philip, *J. Immunol.*, 1926, xii, 441.

[11] Ashby, W., *J. Exp. Med.*, 1919, xxix, 267.

[12] Unger, L. J., *J. Am. Med. Assn.*, 1921, lxxvi, 9.

[13] Guthrie, C. G., and Pessel, J. F. P., *Bull. Johns Hopkins Hosp.*, 1924, xxxv, 33, 81, 126.

[14] Jones, A. R., and Glynn, E. E., *J. Path. and Bact.*, 1926, xxix, 203.

[15] Landsteiner, K., and Witt, D. H., *J. Immunol.*, 1926, xi, 221.

4005

A New Liver Function Test.

ALICE R. BERNHEIM. (Introduced by Ralph G. Stillman.)

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In a number of phenoltetrachlorphthalein liver function tests, determinations of the bilirubin content of the blood showed a rise of the icterus index following the intravenous injection of the dye. When bromosulphalein was used no rise in the index took place. At first the reason for the rise in index following the use of the phenoltetrachlorphthalein was thought to be its toxic action upon the liver. Dr. S. M. Bassett, however, suggested that, inasmuch as distilled water was used to dilute a 5% to a 1% solution in the case of phenoltetrachlorphthalein, and no dilution was made in the case of bromsulphalein, the increase in icterus index might be due to the laking of red blood cells by the distilled water. Accordingly injections of distilled water up to 100 cc. were given intravenously, and, as surmised, a rise in the icterus index was produced.

From this observation the idea arose that this use of distilled water might serve as a liver function test. The rationale was as follows: if the normal liver removes bilirubin from the blood (the source of bilirubin being the hemoglobin from destroyed red blood cells), it is reasonable to suppose that a disordered liver will remove it less efficiently. If, then, in the normal person after the injection of distilled water the icterus index rises from 2 to 4 points one half hour to an hour after injection, with an average rise of 2.5 points, and returns to its basal value in 5 hours, a greater increase in icterus index might conceivably be expected in the individual with a disordered liver, or the return to normal might be prolonged.

Adequate dosage of the water was ascertained by experiment. 25 cc. are used in the case of individuals weighing from 100 to 110 pounds, with an increase of 5 cc. for every pound of body weight up to a maximum dosage of 50 cc.

In the normal person results were obtained as stated. But contrary to expectations the injection of the water in abnormal cases occasioned either no rise in the index or a fall, according to the amount of dysfunction. The interpretation of these observations for the present lies in the realm of speculation. It seems unlikely that a disordered liver can more readily remove the bilirubin from the blood than a normal one. The results indicate that the test may be a measure of the bilirubin-forming, rather than of the bilirubin-removing function of the liver.

Observations were made on 15 normal, healthy persons and on 122 hospital patients. The normal cases showed a rise of from 2 to 4 points in the icterus index. Of the hospital cases 28 showed either no rise or a fall. These 28 cases comprised: 5 of catarrhal jaundice, 1 of arsenical poisoning (with jaundice), 2 of carcinoma of the stomach (with liver metastases), 5 of cirrhosis of the liver, 1 of cyst of the liver (liver enlarged to umbilicus), 1 of Wilson's disease (enlarged liver and spleen), 1 of small liver, right lobe missing (splenectomy for anemia and asthenia), 3 of carcinoma of the head of the pancreas, 3 of cardiac disease with decompensation, 4 of Hodgkin's disease, 2 of Graves disease.

The remaining 94 cases showed a rise in the icterus index. In 5 of these the diagnosis of liver disease was made: 2 cases with the clinical diagnosis of cirrhosis with ascites (both with accompanying kidney lesions), 2 cases of enlarged liver in patients with syphilis, 1 case of localized carcinoma of the liver (extension from gall bladder as shown on operation). Among these some indexes rose less than 2 points.

Cases with the severest liver damage showed a fall in index, those with less damage showed no rise, and normal cases showed a rise of from 2 to 4 points. These facts make it seem possible that in some of the hospital cases showing a rise in index of less than the normal 2 points a correspondingly slight liver dysfunction may be indicated. In 4 cases of thyroid disease (1 adenoma with symptoms of intoxication and 3 of Graves disease) changes in the icterus index following the injection of distilled water corresponded to the rate of basal metabolism and the condition of the patient. The case with a basal rate of plus 31% showed a rise of 1.5 points, that with a rate of plus 40% showed a rise in index of 0.8 points, that with a plus 51% showed no rise, while the 4th case with a plus 80% showed a fall in index of 1.1 points.

Bromosulphalein tests were made to check the water test. Where findings were normal in the dye test they were likewise normal in the water test. With the bromosulphalein test in cases of jaundice there is always a 100% retention of the dye one half hour after injection irrespective of the severity of the liver involvement. With the water test the index either does not change or decreases according to the extent of damage. This test is accordingly a more delicate gauge of disturbance than the dye test, when jaundice is present.

There is a fairly common impression that the intravenous injection of distilled water may produce unpleasant consequences varying from a mild chill to death. In none of these cases were there any unpleasant reactions. On the contrary, restless patients felt quieted and all without exception experienced a feeling of well-being lasting several hours.

4006

Sulphur Partition of Ocular Humors and Presence of Glutathione in Lenses of the Eye.

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Gravimetric determinations of total sulphur, non-protein sulphur, total sulphates and inorganic sulphates have been made upon the humors of oxen eyes. Mean figures are based upon from 6 to 14 analyses in each instance. The total sulphur of the aqueous humor was 3.74 mg. per 100 cc., and of this 64% was found to be nonprotein sulphur. Sulphate sulphur formed 54% of the nonprotein sulphur. Ninety % of the total sulphates were present as inorganic sulphates.

In the vitreous filtrate 4.05 mg. per 100 cc. of total sulphur were found, and of this total sulphur, 54% was non-protein sulphur. Seventy-four % of the nonprotein sulphur was sulphate sulphur. The inorganic sulphates formed 83% of the total sulphates.

The sodium nitroprusside test for glutathione has been applied to normal lenses, both human and oxen, and to cataractous lenses. All of the normal lenses gave a deep magenta color. Of 64 cataractous lenses, 26 (40%) showed no color, 19 presented a trace of color about the periphery, and in 19 a color definitely lighter than in the normal lenses was obtained.

4007

A Scopometric Method for the Estimation of Sugar in Urine.

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The mercuric atom of the double mercuric-sodium chloride salt in carbonate solution is reduced by glucose at room temperature, giving a fine crystalline precipitate of mercurous chloride. This precipitate bears a quantitatively reproducible relation to the concentration of glucose which can be easily measured in the Junior Scopometer.[1] With low concentration (circa. 5 mg. glucose per cc.) the turbidity is equivalent to 8 scale divisions per mg. on a wedge calibrated for urinary proteins precipitated by Exton's reagent and with high concentration (100 mg.) somewhat less than one scale division. This gives a wide range in its practical application and sensitiveness where this is most important for clinical interpretation.

The microscopic crystalline precipitate consists of stubby needles when precipitated from a pure solution in the presence of certain colloids. Without the colloid the needles form crosses, and in the presence of acetate ions these fill out giving square plates with cross figures. The crystal habit and size are reasonably controllable even in urinary filtrates. Other substances also reduce the mercuric atoms. Of those which may be present in urine the volatile aldehyde and ketones reduce the mercury before the solution is made alkaline and the mercurous chloride from this source may be removed before the glucose reduction is initiated by the addition of carbonate.

Creatinine and many other substances are removed by a preliminary clarification. Mercuric salts have been found convenient for this purpose, each salt and combination of salts removes most of the urinary constituents in definite fractions. This matter is being investigated in detail and will be the subject of subsequent contributions since it is considered of practical value in clinical chemistry.

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[1] Exton, W. G., *J. Lab. Clin. Med.*, 1928, xiii, 385; *Arch. Path. Lab. Med.*, 1928, v, 49.

4008

The Grading of Collodion Membranes by Means of Ethylene Glycol.

ADA M. FIELD AND ARTHUR W. THOMAS.

From the Department of Chemistry, Columbia University.

In the ethylene glycol method of grading collodion membranes[1] the grading is brought about not, as Pierce supposed, by ethylene glycol alone but by ethylene glycol plus the alcohol retained in the membranes after 24 hours drying.

Ethyl alcohol is readily absorbed both by ethylene glycol and by calcium chloride, therefore the amount of alcohol retained by the membrane is determined in part by the per cent of glycol in the collodion solution employed, and in part by (1) the quality of calcium chloride used in the outlet from the drying chamber and (2) the accessibility of this chloride to the alcohol vapors from the chamber. By varying the diameter of the opening between the floor of the drying chamber and the container of calcium chloride, wide variations were produced in the "weight before washing" and in the "wet weight" of membranes prepared from collodion solution of the same ethylene glycol content, while with the calcium chloride constant in all respects the grading by means of ethylene glycol was good.

4009

Experimental Ulcer of the Colon in Rats.

W. M. BALDWIN.

From the Department of Anatomy, Albany Medical College.

This work is part of a series of experiments to determine whether subcutaneous injections of Mercurochrome-220 Soluble (Hynson, Westcott and Dunning, Baltimore, Md., U. S. A.) rendered adult white rats more susceptible to the lethal effects of X-ray energy. On alternate days, 1.0 to 2.0 cc. of a 0.5% solution in normal saline was injected into the dorsal subcutaneous tissue of 12 adult rats, all of the same size. Different areas were selected for the successive injections to avoid a possible necrosis of the tissues from too concentrated and prolonged action of the stain. Thus each rat received a total of from 6.0 cc. to 8.0 cc. of this solution. Upon the 4th day, there was noticeable a distinct reddish tinge to the skin, ears, eyes, mucous membranes, and to the feces and urine as well. All of the animals were killed after the 4th injection; *i. e.,* 7 days after the beginning of the experiment.

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[1] Pierce, H. F., *J. Biol. Chem.*, 1927, lxxv, 795.

At autopsy, the connective tissues, fat, blood, skin and various organs of the digestive tract and of the urinary tract were distinctly stained with the dye, as has been reported by other investigators.[1]

Upon microscopic examination, the kidneys presented histologic changes more marked in the glomeruli and in the cells lining the convoluted tubules. The vessels of the glomeruli and of the adjacent portions of the tubules were distended and filled with deeply stained red blood cells. The columnar lining of the convoluted tubules presented various stages of cloudy swelling, necrosis, and exfoliation. The most interesting and most constant of the histologic findings were referable, however, to the cecum and ascending colon.

Young and Hill[1] have reported colitis and enteritis present in animals receiving intravenous therapy, and, likewise, Todd[2] has recognized a form of catarrhal colitis. Such findings have been verified by this experiment. And, in addition, a constant ulcer was identified upon the median wall of the ascending colon, directly cephalad to the iliocecal valve. This ulcer was circular in outline, averaging from 3.0 to 5.0 mm. in diameter, with a depth varying from a superficial erosion, involving epithelium alone, to more extensive necrosis comprising both mucosa and submucosa, and with a base formed by the circular muscle fibers of the gut wall. In no instance were these muscle fibers involved in the ulcer. The ulcer crator was filled with clotted blood, tissue detritus and intestinal content, all deeply stained with mercurochrome. The edges of the ulcer were sharply defined and presented some degree of edema with cellular infiltration of the adjacent tissues. Various stages of degeneration and necrosis of these tissues, dilated capillaries choked with stained red cells and a certain degree of leukocytosis constituted the readily recognizable essential features.

The constant location of this ulcer is the most significant single feature of the histologic findings and has been hitherto unreported.

Evidence has been presented by the authors above cited that mercurochrome is excreted through the colon mucosa. While it is possible to imagine that the excretion of a considerable amount of this drug in sufficient concentration might be the direct cause of the primary irritation which subsequently resulted in a necrosis of the mucosal lining, it is difficult to conceive of a sharp restriction of this excretory function to only those cells of the colon mucosa which occupy the position of the ulcer. No other colon ulcer was found in this series. On the other hand, it is not unreasonable to assume that mercurochrome is excreted through the small intestine mucosa even though the experimental evidence for this feature is difficult to obtain. Certainly the enteritis which has been reported by Young and Hill is explicable upon this basis. Assuming that a certain amount of mercurochrome well diluted is excreted through the small intestine mucosa, the rapid absorption of fluid from this intestinal content upon arrival and stasis in the caecum might well increase the concentration of the chemical to the degree that its corrosive action becomes manifest upon that portion of the colon mucosa where that concentration is greatest. This is the only hypothysis which appears plausible to the mind of the author.

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[1] Young and Hill, *Arch. Pediat.,* 1927, xliv, 152.

[2] Todd, *Lancet,* November 14, 1925.

4010

Response of High Protein Diets and Unilateral Nephrectomy During Reproduction in the Rat.*

HELEN T. PARSONS,† ARTHUR H. SMITH AND T. S. MOISE.

From the Laboratory of Physiological Chemistry and the Department of Surgery, Yale University.

This study was undertaken to determine the extent to which the task of excretion of nitrogenous katabolites could be intensified in the organism by simultaneous reduction of kidney tissue, reproduction with lactation and high protein intake and to observe the physiological adjustments made to these conditions. Unilateral nephrectomy was performed on adult female rats which were placed immediately on the experimental rations and, as soon as the regular oestrous cycle was reestablished, were mated. The weight of the litters at birth and at weaning were compared and lactation closely observed during this time. Some of the mother rats were killed after the first litter was weaned and others were bred one or more times further, and then killed.

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* Part of the expenses of this investigation was defrayed by grants from the American Medical Association and from the Russell H. Chittenden Fund for Physiological Chemistry.

† Mary Pemberton Nourse Fellow of the American Association of University Women.

Reproduction on the protein diets was fairly successful but lactation was definitely deficient until certain changes were made in the experimental food. In Table I is given a summary of the high protein rations and control low protein rations fed.

TABLE I.
Summary of Basal Diets.

High Protein

Ingredients	Range of percentages of ingredients in the group of high protein rations	
	%	%
Casein*	72-86	
Egg albumen (cooked and dried)		72-86
Salt mixture (Osborne and Mendel)	4	4
Added $CaCO_3$	0-4	0-4
Butter fat	0-10	0-10
Wheat embryo	0-20	0-20

Low Protein

Ingredients	Range of percentages of ingredients in the group of low protein rations	
	%	%
Casein*	20	
Egg albumen (cooked and dried)		20
Cooked starch	54-66	54-66
Salt mixture (Osborne and Mendel)	4	4
Added $CaCO_3$	0-1.67	0-1.67
Butter fat	0-10	0-10
Wheat embryo	0-20	0-20

Daily Additions to Basal Ration

Yeast*	1-3 gm. (depending on the period in reference to reproduction)
Cod liver oil	0-1½ cc.
Lettuce	0-15 gm.

* Portions of either yeast or casein were irradiated for each ration.

Renal enlargement calculated on the basis of increase in weight of the remaining (left) kidney over the "normal" kidney weight corresponding to the actual heart weight ranged from 219 to 29% in the animals on the protein-rich rations and from 58 to 7% in those on the low-protein control food. The degree of renal enlargement appeared to be due largely to 2 factors, namely, to the concentration of dietary protein and to the extent to which the maternal organism was called upon to satisfy the nutritional demands of the suckling young. The length of time on the ration after nephrectomy, the age of the animal, and gestation unaccompanied by lactation, were comparatively negligible factors in the renal enlargement under the conditions of these experiments.

Blood urea determinations showed that in the nephrectomized control mother rats the values rose from a normal of 23-44 mg. per 100 cc. to 60-70 mg. during lactation, while the urea concentration for the rats on the high protein ration rose from the "normal" to 66-130 mg. after nephrectomy during pregnancy and to a content as high as 188 mg. during lactation. These high values were not due to changes in blood concentration in general.

A few of the rats which were subjected to the greatest reproductive strain and which were given the high protein ration for periods varying from 106 to 187 days, showed renal lesions comparable to those described by Moise and Smith.[1]

4011

Further Studies on the Life History of Blepharisma Undulans.

LORANDE LOSS WOODRUFF.

From the Osborn Zoological Laboratory, Yale University.

Studies on the life history of *Blepharisma undulans* were carried on for just 6 years, all the animals employed being the progeny of a single individual isolated November, 1921. The work was planned to determine, in particular, the viability of this infusorian in the absence of fertilization, and the effects of fertilization on the rate of reproduction, in an attempt to broaden the results from our earlier studies on *Paramecium aurelia*[1] and *Spathidium spathula*.[2] A preliminary statement of the characteristics of this race of *Blepharisma undulans* was published in 1922,[3] and a summary of the chief experimental results was presented in 1927.[4]

The present communication is to reiterate the results previously presented in the light of the final year of study of the culture.

In brief, the conclusions derived from *Paramecium* and *Spath-*

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[1] Moise, T. S., and Smith, A. H., *J. Exp. Med.*, 1927, xlvi, 27.

[1] Woodruff, L. L., *Quar. Rev. of Biol.*, 1926, i, 436.

[2] Woodruff, L. L., and Spencer, PROC. SOC. EXP. BIOL. AND MED., 1921, xviii, 240, 303; *J. Exp. Zool.*, 1924, xxxix, 133.

[3] Woodruff, L. L., and Spencer, PROC. SOC. EXP. BIOL. AND MED., 1922, xix, 339.

[4] Woodruff, L. L., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 769.

idium that these infusorians are capable of unlimited reproduction, under favorable environmental conditions, without recourse to fertilization, may now be extended to *Blepharisma;* and, furthermore, the conclusion that fertilization accelerates the division rate, as previously demonstrated in *Uroleptus*[5] and in *Spathidium,*[2] may now be extended to *Blepharisma.*

These studies have been aided by a grant from the Bache Fund of the National Academy of Sciences.

4012

An Experimental Study on Formation of Middle Ear in Rana.

H. N. VIOLETTE. (Introduced by J. S. Nicholas.)

From the Osborn Zoological Laboratory, Yale University.

The otic vesicle of various species of Rana was extirpated completely and in part. In one series the ear was grafted homoplastically to the flank, and later regrafted to the orthotopic position. The animals were carried through metamorphosis, to study the effects of these operations upon the formation of the middle ear. It was sought to determine the extent which differentiation of the inner ear influences the formation of the plectrum and tympanum, and the periods at which this influence may be effective.

The operations were performed on the left ear. Equilibration was found to be abnormal, the larvae swimming in counter-clockwise spirals or in clockwise circles. The larvae at rest were found to lie upon the left side. Normal equilibration was regained in some of the older larvae, but a number of metamorphosed animals showed a sharp inclination of the head to the left.

The grafted ear vesicle differentiated more or less completely. In some cases considerable differentiation of the otoliths took place. Normal differentiation of the Anuran ear in heterotopic position has recently been described.

The metamorphosed animals had a fully developed tympanic membrane on the operated side. The *annulus tympanicus* was present. A plectrum was formed when but a small rudimentary ear was present, its medial end fused with the ear capsule, which showed no operculum. The tympanum and Eustachian tube were formed normally.

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[5] Calkins, G. N., *J. Exp. Zool.*, 1919, xxix, 121.

4013

Normal Survival Period Following Bilateral Epinephrectomy in the Cat.

E. L. COREY. (Introduced by J. S. Nicholas.)

From the Osborn Zoological Laboratory, Yale University.

Marked discrepancies in regard to the normal survival period in cats, following bilateral adrenal extirpation, are apparent throughout the literature dealing with the adrenal cortex. Zwemer[1] states the average survival period for a series of 43 cats to be 53 hours with 26 and 111 hours as the extremes. Swingle[2] finds the average survival period for bilaterally epinephrectomized cats to be about 60 hours. Houssay and Lewis[3] found that animals from which the last vestige of cortical tissue had been removed survived but from 24 to 26 hours. Hartman,[4] in seeking an active cortical extract, considered 90 hours as a marked prolongation of the life of bilaterally operated cats. The above references will serve as evidence that no definite criterion has as yet been established in work of this nature.

In a series of 31 animals the author has found the average survival period to be 115 hours, and concludes that the normal life-span of untreated bilaterally epinephrectomized cats must be placed slightly in excess of 100 hours. These findings have been completely corroborated by the recent work of other investigators.

Hartman[5] has placed the average survival period at from 5 to 6 days. Marine and Bauman[6] also agree that the life-span should be placed at somewhat in excess of 100 hours (5.3 days). Professor Swingle, in recent communications to the author, states that he would place this period in excess of 100 hours. Zwemer, in a recent conference with the writer, has stated that he would at present place the life-span of control animals at approximately 100 hours.

The author is inclined to attribute the comparatively short survival periods obtained by various workers to imperfections in operative technique, excessive visceral manipulation during operation, or to excessive handling and excitation.

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[1] Zwemer, R. L., *Am. J. Physiol.*, 1927, lxxix, 641.

[2] Swingle, W. W., *Am. Nat.*, 1927, lxi, 132.

[3] Houssay, B. A., and Lewis, J. T., *Am. J. Physiol.*, 1923, lxiv, 512.

[4] Hartman, F. A., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 467.

[5] Hartman, F. A., MacArthur, C. G., and Hartman, W. E., PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 69.

[6] Marine, D., and Bauman, E. J., *Am. J. Physiol.*, 1927, lxxxi, 86.

The establishment of the normal survival period of bilaterally epinephrectomized cats is of prime importance, since the efficacy of all treatment devised to prolong life, as well as the potency of extracts obtained from the adrenal cortex, can only be interpreted by comparison with a definitely established normal survival period.

4014

Growth of Heteroplastically Transplanted Eyes and Limbs in Amblystoma.

VICTOR C. TWITTY AND JOSEPH L. SCHWIND.
(Introduced by J. S. Nicholas.)
From the Osborn Zoological Laboratory, Yale University.

For the purpose of comparing the growth of normal and heteroplastically transplanted organs, eye vesicles (with lens ectoderm) and limb discs were transplanted between *Amblystoma punctatum* and *A. tigrinum*. The operations were done in the "tail-bud" stage, and the grafts were placed in the normal position and orientation. To standardize the feeding, both species were daily fed the maximum amount they would eat.

Measurements of normal and transplanted eyes were made with a micrometer ocular at regular intervals. As accurate measurements of the developing limb are impossible, pairs of animals with heteroplastically transplanted limbs were killed from time to time as a comparison.

Measurements made as soon as the outline of the eye became sufficiently visible, before either species had reached the feeding stage, showed that the normal and transplanted eyes were of the same size. This relation is maintained during most of the larval period. As the time of metamorphosis approaches, accurate measurements of the eye can no longer be made, but apparently the normal *tigrinum* eye now becomes slightly larger than the transplanted eye on the *punctatum*, and the transplanted *punctatum* eye becomes larger than its control. In other words, the 2 eyes on the *tigrinum*, normal and transplanted, become larger than the 2 on the *punctatum*.

In normal development, the fore-limb of *Amblystoma punctatum* becomes visible externally about the same time as the gills, while that of *A. tigrinum* does not appear until just before the animal begins to feed. When the limb disc is transplanted heteroplastically, the transplanted limb and the normal limb of the donor species appear at the same time. During the major portion of larval life the transplanted limb and the normal limb of the donor species maintain the same size. Eventually, in late larval life, the normal limb of the *tigrinum* becomes somewhat larger than the limb transplanted to the *punctatum,* and the transplanted *punctatum* limb becomes larger than its control. The growth relations of the eye and limb after heteroplastic transplantation are therefore the same.

There is a great difference in the growth rate of the 2 species. With maximum feeding, 5 weeks after operation the *tigrinum* has attained a length twice that of the *punctatum,* and therefore requires much more food. We have found that if this greater food requirement of the *tigrinum* is not met, its growth will be correspondingly retarded, and the normal *tigrinum* organ will be smaller than that transplanted to *punctatum,* and conversely the *punctatum* organ on *tigrinum* will not become as large as the normal control on *punctatum.*

With maximum feeding, then, the behavior of heteroplastically transplanted organs indicates that the factors controlling their growth are intrinsic to the grafted tissue.

4015

Anhydraemia in Rats Suffering From Lack of What Has Been Called Vitamin B.

W. B. ROSE and C. J. STUCKY. (Introduced by George R. Cowgill.)

From the Laboratory of Physiological Chemistry, Yale University.

Students of vitamin "B" deficiency are well aware that animals fed a complete diet except with respect to vitamin "B" lose their appetite for the deficient ration and rapidly decline in body weight. It is also well known that an increase of blood concentration occurs in starvation. The question arises whether a deficiency of what has been spoken of hitherto as vitamin B is associated with anhydraemia, and if such is the case, whether this can be accounted for by the starvation phenomenon which so frequently prevails.

Consequently, a series of albino rats weighing between 200 and 250 gm. were placed on a vitamin "B" deficient diet. Control animals were given on an average the same amount of food and water daily as the vitamin "B" deficient rats consumed. Blood samples were obtained by cardiac puncture from each rat of both groups from week to week during the course of the vitamin deficient and realimentation periods. Hemoglobin estimations were made by the Cohen and Smith[1] method.

The results indicate that a definite increase in hemoglobin occurs during the vitamin "B" deficient period followed by a very distinct drop during the first few days of realimentation. The parallel changes that developed in the control group of rats indicate that the phenomenon just described may be accounted for by the diminished food and water consumption incident to "B" avitaminosis. Other experiments performed on another species (dog) and extending over a period of approximately 1 year appear to confirm these results.

4016

Gastric Motility in Relation to Anhydraemia.

W. B. ROSE and C. J. STUCKY. (Introduced by George R. Cowgill.)
From the Laboratory of Physiological Chemistry, Yale University.

In an attempt to throw some light on the disturbances of gastric motility occurring during the deficiency of what has been spoken of hitherto as vitamin B these experiments were undertaken. Dogs were deprived of water and parallel observations on gastric motility, and blood concentration as indicated by hemoglobin estimations, were made. Except for anorexia and decrease in general activity no abnormal symptoms were in evidence. All of the dogs developed an increase of approximately 20% in hemoglobin (estimated by the method of Cohen and Smith[1]) and complete gastric atony. Liberal administration of water produced a rapid decrement in blood concentration, followed by a return of gastric motility to normal within a few days. The results indicate a striking parallelism to exist between anhydraemia and gastric motility. The significance of this phenomenon in relation to disturbances of gastric motility occurring in "B"-avitaminosis will be discussed elsewhere.

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[1] Cohen, B., and Smith, A. H., *J. Biol. Chem.*, 1919, xxxix, 489.

[1] Cohen, B., and Smith, A. H., *J. Biol. Chem.*, 1919, xxxix, 489.

4017

Preparation of Relatively Stable Emulsions With Kahn Antigen Base.

B. S. KLINE.

From the Laboratory Department, Mount Sinai Hospital of Cleveland.

In a previous article[1] describing a purified antigen for serum tests for syphilis, a formula was given for the preparation of most satisfactory precipitation test emulsions that retained their antigenic properties undiminished for 3 days. A further study of the Kahn antigen base has shown that by the following procedures, relatively stable emulsions can be prepared from it also:

The Kahn antigen base described by Kahn[2] (non-cholesterinized alcoholic extract of heart muscle powder previously extracted with ether) is evaporated at 45° C. to 50° C. on water bath (thermometer bulb within the extract). The resultant wax is weighed and 10 cc. of hot (at 50° C.) absolute alcohol (99+%) is added to each gram in glass stoppered bottle, shaken about 5 minutes, and the turbid solution placed in incubator at 50° C. for 1 hour. Bottle is shaken a few times to promote solution.

The turbid solution is then placed in refrigerator at 8° C. for 4 to 5 hours during which a whitish precipitate settles out. This is filtered off and the resultant filtrate is the antigen. The same formula as previously described[1] is then used as follows:

1. *Very Sensitive Antigen Emulsion.*	2. *Sensitive Antigen Emulsion.*
Distilled water, 0.85 cc.	0.85 cc.
Cholesterin (Merck)* 1.25 cc.	
of 1% sol. in	0.75 cc.
absolute ethyl alcohol	Same.
Antigen (concentrated, chilled Kahn extract), 0.1 cc.	Same.
NaCl (C. P. Merck), 2.2 cc. of 0.85%.	2.75 cc.

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* The 1% cholesterin solution is prepared as follows: To 1 gm. of cholesterin (Merck), 100 cc. absolute ethyl alcohol is added and the bottle placed in an incubator at 50° C. to 56° C. The bottle is shaken at 15 minute intervals to promote solution (which is complete) in about 45 minutes. This stock solution is kept in the incubator at 37° C. and is perfectly satisfactory for the preparation of the emulsions for as long as 2 months.

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[1] Kline, B. S., *J. Lab. and Clin. Med.*, 1928, xiii, 588.

[2] Kahn, R. L., 'Serum Diagnosis of Syphilis by Precipitation.'' Williams & Wilkins Co., 1925, 132.

The antigen emulsions are prepared as follows. Into 1 ounce bottle 0.85 cc. of distilled water is pipetted. The bottle is held at an angle and the 1% cholesterin (Merck) in absolute ethyl alcohol is allowed to run along the side of the bottle. The bottle is gently rotated from the neck for from 15 to 20 seconds. The bottle is held at an angle again and 0.1 cc. of antigen is pipetted against the side from a 0.2 cc. pipette (graduated in thousandths). The bottle is promptly stoppered with a cork covered with tin foil and shaken fairly vigorously (the fluid thrown from the bottom to cork and back) for 30 seconds. Lastly, the 0.85% sodium chloride (C. P. Merck) solution is allowed to run in quite rapidly, the bottle is stoppered again and shaken as previously for 30 seconds.

The emulsions, when examined through the microscope at a magnification of about 75 times, show numerous exceedingly fine particles. They are satisfactory for use in precipitation tests immediately after preparation and at any time within 3 days (kept at room temperature).

The formula given above is based upon the following observations: Uniformly satisfactory emulsions result only when the cholesterin is precipitated before the antigen is added. Furthermore it has been found that the sensitivity of the precipitation test depends more upon the quantity of cholesterin than of the antigen present. Again, it was observed that alcohol interferes with the stability and cholesterin dispersion of antigen emulsions. This explains the use of a much more concentrated antigen than employed by other workers. As a result, the emulsions are relatively stable and as more cholesterin may be dispersed in a given total of emulsion, greater sensitivity is achieved. When prepared by the formula outlined above the antigen emulsions are superior to the Kahn antigen dilutions made from the same extract in the following ways:

Antigen Emulsions (Kahn Ext.)	*Kahn Antigen Dilutions.*
(a) Relatively stable, satisfactory for at least 3 days. (After this, at room temperature, emulsions are still exceedingly finely granular when examined through the microscope, magnification about 75 times, and contain no clumps or crystals.)	(a) Unstable, satisfactory for an hour and a half at most, usually less.
(b) May be used immediately after preparation.	(b) Must stand from 10 to 15 minutes before use.
(c) Satisfactory at low room temperatures as well as at ordinary ones.	(c) Majority unsatisfactory at low room temperatures.

(d) The very sensitive emulsion is more sensitive than the Kahn antigen dilution from the same extract and no less specific.

In addition it has been found that a Kahn extract giving unsatisfactory antigen dilutions (because of small granular particles not disappearing with greater saline dilution) yields satisfactory emulsions when concentrated, chilled and mixed as described above.

The following charts illustrate the advantages of the emulsions of modified Kahn antigen base in comparison with Kahn antigen dilutions from the same extract:

CHART I.

Comparison of stable antigen emulsion and Kahn antigen dilution in the microscopic slide precipitation test for syphilis.[3]

	1	2	3	4
			Sera retested 3 days later with	
Number of sera tested	Freshly prepared emulsion of concentrated chilled Kahn extract No. 4 by new formula	Freshly prepared Kahn antigen dilution (same extract as 1, 3, 4)	Three day old emulsion of concentrated chilled Kahn extract No. 4 by new formula	Freshly prepared emulsion of concentrated chilled Kahn extract No. 4 by new formula
11	—	—	—	—
1	+, ++	++	+, ++	+, ++
1	++	++	++	++
1	++	++	+	+
1	++	+	+	+, ++
1	+++	+++	++, +++	+++
3	++++	++++	+++, ++++	++++
3	++++	++++	++++	++++

Total sera tested, 22.

Tests for syphilis with the emulsions of Kahn extracts prepared as described in this paper, agreed closely with those in which a purified antigen was used and with Wassermann tests (Cleveland method) in which a cholesterinized antigen was employed. The preparation and necessary modification of the Kahn antigen base for most advantageous use is more laborious than the preparation of a purified antigen previously described.[1]

Conclusions. 1. Kahn antigen base when concentrated, chilled and used according to a formula described above yields thoroughly satisfactory emulsions that retain their antigenic properties undiminished in precipitation tests for syphilis for 3 days.

2. These emulsions are further superior to Kahn antigen dilutions in tests done at low room temperatures in that under these conditions they give no false clumping.

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[3] Kline, B. S., and Young, A. M., *J. Lab. and Clin. Med.*, 1927, xii, 477; *Am. J. Syphilis*, 1927, xi, 290.

CHART II.
Comparison of stable antigen emulsion and Kahn antigen dilution in the microscopic slide precipitation test for syphilis.

Number of sera tested. Courtesy of Dr. H. J. Knapp, Cleveland Health Department	1 Three day old emulsion of concentrated chilled Kahn extract No. 4 by new formula	2 Freshly prepared emulsion of concentrated chilled Kahn extract No. 4 by new formula	3 Freshly prepared Kahn antigen dilution (same extract as 1 and 2)	4 Three day old emulsion of concentrated chilled Kahn extract No. 1 by new formula	5 Freshly prepared emulsion of concentrated chilled Kahn extract No. 1 by new formula
49	—	—	—		
4	—	—	—	—	—
1	±	—	—	—	—
1	±	±	—	—?	±
1	±	±	—	—	—
2	±, +	±	—	—	—
1	+	±	±	—	—
1	++	++	++	++	++, +++
1	++	+	+	++	+, ++
1	+++	++	—?		++
1	++++	++++	++++	+++	++, +++
3	++++	++++	++++		
7	++++	++++	++++	++++	++++

Total sera tested, 73.

3. Because more cholesterin may be dispersed in a given total by the new formula, it is possible to make the emulsions more sensitive, yet no less specific, than Kahn antigen dilutions from the same extract.

4018

Bronchial Perfusion of Isolated Lung as a Method for Studying Pharmacologic Reactions of Bronchiolar Muscle.

TORALD SOLLMANN AND W. F. VON OETTINGEN.

From the Department of Pharmacology of the School of Medicine of Western Reserve University.

The methods for studying the changes of bronchiolar tone in intact animals are often complicated by changes in the circulation which make their interpretation uncertain. It would therefore be desirable to check them under conditions in which the circulation would be excluded, namely outside of the body. The use of ring preparations of tracheal or bronchial muscle for this purpose has not been altogether satisfactory, partly because these are confined to the trachea and larger bronchi which might react differently from the smaller bronchioles that play the major part in the bronchial reactions; and partly because it is difficult to reproduce the natural conditions of tension. These objections are avoided in the following method, which is based on measuring the rate of flow of a Locke solution through the bronchial tree, allowing the fluid to drain off by filtration.

The arrangement is shown diagramatically in Fig. 1. A Mariotte bottle (M) filled with Locke solution is connected with a Woulff bottle (W), also filled with Locke solution, which sits in a bath (B) regulated to keep the temperature of the solution at 39° to 40° C., as indicated by the thermometer (Th). The outflow passes to a ⊣ tube (T). The upper limb of this is furnished with a short piece of rubber tubing (R) which may be closed by a screwclamp (S). The lower limb is connected with a cannula tied into the short stump of the trachea of the excised lung (L). This should be used either promptly after the death of the animal, or else after lying on ice.

In starting the experiment, the T tube is raised so that no fluid flows from the Mariotte bottle; the screwclamp (S) on the upper limb is opened, and the lung is attached to the lower limb. The T is then gradually lowered so that just enough liquid flows into the bronchi to distend the lung approximately to its normal size. The lung is then gently squeezed to expell the air through the vertical limb. When foam ceases to rise, the screwclamp is closed and the height of the lung is adjusted so that the air enters the Mariotte bottle at the rate of about 20 bubbles per minute. When the rate of bubbling has become nearly constant, indicating that the volume of the lung has become adjusted to the pressure of the system, the preparation is ready for the experiments.

The drugs are injected in 1 cc. of solution from a syringe into the rubber tube, between the T and the lung; followed immediately by the withdrawal of the same volume of fluid, through the tubing near the Woulff bottle, to balance the changes of pressure in the system. The rate of flow should not be estimated by the outflow of liquid from the lungs, since this is complicated by the alterations of capacity through edema and stretching. It should be measured by the entrance of air into the Mariotte bottle; either simply counting the number of bubbles per minute by a watch; or connecting the Mariotte bottle through Ta to a tambour registering on a drum, directly or through an electric relay. However, such registration is generally a needless complication.

Figure 2 illustrates the results obtained with this method. In Fig. 2A 1 cc. of pilocarpine, 1:1000, was injected into the trachea,

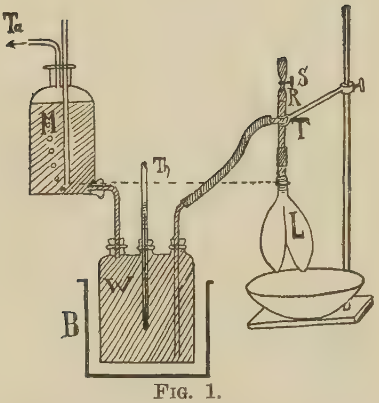

FIG. 1.
Diagram of bronchial perfusion. The letters are explained in the text.

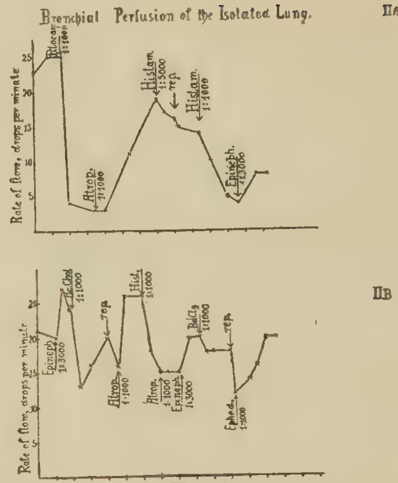

FIG. 2.
Charts of two experiments, illustrating the effects of drugs on the bronchial flow. The solutions were always injected in the volume of 1 cc. The time is marked in 5 minutes. "Rep."—repetition of injection.

which was immediately followed by a reduction of the inflow from 25 to 4 bubbles. About 10 minutes later 1 cc. of atropine sulphate, 1:1000, was injected, which produced a dilation indicated by an increase from 3 to 19 bubbles within 15 minutes. 1 cc. histamine, 1:5000, produced a slight constriction which became more pronounced by repeating the injection with a solution of 1:1000, as indicated by a decrease of the inflow from 19 bubbles to 4 within 25 minutes. Epinephrine, 1:3000, after histamine, produced only a slight dilation characterized by an increase from 4 to 8 bubbles per minute.

Fig. 2B illustrates a similar experiment. 1 cc. of epinephrine, 1:3000, produces a short increase of the inflow of 35%, acetylcholine, 1:1000, reduces it by 35%. Atropine, 1:1000, after acetylcholine produces dilation above the normal, and histamine, 1:1000, produces a rather marked constriction of 55%. Atropine, 1:1000, after histamine shows no effect, whereas epinephrine, 1:3000, produces a slight dilation. Barium chloride, 1:1000, produces another constriction of 40%, which is abolished by the subsequent injection of epinephrine, 1:1000.

As may be seen from these experiments, the method permits the study of the responses of the bronchial muscle to autonomic and muscular poisons, and gives a clear-cut picture of their action, without the interference of changes of the circulation or of the volume of the heart.

4019

A Device for Determining Refractory Period of the Mammalian Heart During Normal Sinus Rhythm.

HAROLD A. WHEELER AND E. COWLES ANDRUS.
(Introduced by Edward P. Carter.)

From the Department of Physics, Johns Hopkins University, and the Cardiographic Laboratory, Johns Hopkins Hospital and University.

In previous determinations of the refractory period of the mammalian heart an artificial rhythm has been established by means of a series of break induction shocks at a rate necessarily more rapid than that of the normal sinus rhythm. This rhythm has then been interrupted by break shocks timed by some mechanical device to fall at various points in diastole. Such measurements have, therefor, yielded the refractory period of an artificially induced excitation.

The authors have devised an apparatus utilizing the action current after amplification with which it is possible to interrupt the *sinus* rhythm at any interval following a normal excitation. The amplifier is designed to be connected to the terminals of the galvanometer string without affecting the performance of the latter. The electric impulse to be amplified is on the order of 1 millivolt for 1/50 second, and the current supplied to the amplifier must be negligible as compared with about 1 microampere, the galvanometer current. A 5-stage, resistance-coupled amplifier is used, with 4 UX-240 tubes and 1 UX-112A. The string terminals are connected directly in the first grid circuit. The 4 coupling condensers are large, so as to give the entire amplifier a discharging time constant of 1/6 second. The first 2 plate circuits have condensers in parallel, so as to obtain a charging time constant of 1/50 second. In this way, the amplifier is rendered free from impulses of much longer or shorter duration. A 10,000-ohm magnet carries the plate current of the last tube, normally less than ½ milliampere because of a large negative grid voltage. An impulse of ½ millivolt for 1/50 second, applied to the amplifier, is amplified to over 20 volts for 1/10 second at the magnet terminals, about 200,000 times the applied impulse.

The only adjustments in operating the amplifier are: a parallel resistance to adjust the applied impulse to about 1 millivolt, and a reversing switch to secure the correct polarity. A switch in parallel with the magnet, when closed, allows any adjustments while observing the deflections of a milliammeter in series—without actuating the magnet. On the second impulse after opening this switch, the magnet, through an escapement, releases a falling arm which, in turn, opens a trip-switch, breaking the primary circuit of an induction coil. Operation on the second impulse eliminates errors which might otherwise enter if the switch is opened during the first impulse. The trip-switch is opened after a delay of 1/20 to ½ second, depending upon the position of adjustable weights on the falling arm.

4020

Effects of Chicory on the Isolated Intestine.

CHAPMAN REYNOLDS.

From the Department of Pharmacology and Experimental Therapeutics, School of Medicine, Tulane University.

Although chicory is widely used as a beverage ingredient, very little information as to its action is available in the literature. The popular belief, perhaps founded on statements by those commercially interested, that it serves as a mild laxative, suggested testing its effects on isolated intestine. It has been shown that coffee infusions increase the activity of isolated intestinal segments[1] to a greater extent than could be explained by their caffeine content.[2] Kymographic tracings were made from segments of rabbit duodenum in the usual fashion. The preparation tested was a fresh 10% infusion of ground and roasted chicory, made in Tyrode's solution. In numerous experiments we have observed no indication of stimulation of either tone or amplitude of contraction, but with concentrations greater than would be present in the gastro-intestinal tract of individuals drinking chicory-coffee infusions there was uniformly a more or less marked depressant effect.

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[1] Bickel, A., and von Eweyk, C., *Z. f. d. Ges. Exp. Med.*, 1927, liv, 75.

[2] Plant, O. H., and Reynolds, C., *J. Pharm. and Exp. Ther.*, 1922, xix, 256.

4021

Supplementary Values Among Foods. I: Adding Egg to an Adequate Diet Compared with Increasing Vegetables.

MARY S. ROSE AND ELLA L. McCOLLUM.
With the Cooperation of Ruth E. Evans and Emily M. Bloomfield.

From the Institute of Child Welfare Research and the Nutrition Laboratory, Teachers College, Columbia University.

Sherman and Campbell[1] have shown that in a simplified ration composed of whole wheat and whole milk powder marked improvement in growth, reproduction, lactation and general vigor resulted from increasing the milk from 1/6 to 1/3 of the total weight.

We have fed albino rats simplified rations patterned after diets suitable for children, including milk, cereal food (represented by white flour), an assortment of vegetables, a butter substitute (equaling butter in vitamin A content) and meat residue. The composition of these diets is shown in Table I.

TABLE I.
Distribution of Calories in Each Diet.

	H %	M %	L %	E %
White flour	50.0	37.5	25.0	37.5
Milk powder	25.0	25.0	25.0	25.0
Dextrin	5.0	9.5	13.0	9.5
Butter substitute	8.0	10.0	12.0	8.5
Meat residue	2.0	3.0	5.0	1.5
Vegetables*	10.0	15.0	20.0	15.0
Egg	—	—	—	3.0
Total	100.0	100.0	100.0	100.0

* The vegetables used were potatoes, carrots, spinach, tomatoes, onions and peas, all fed in dry form.

On each diet a 5th generation has been secured, but the present report covers only 3 generations; on each diet approximately 40 animals have been carried to the age of 1 year, and on each over 1000 young have been born. As many young as survived have been reared to the age of 28 days. The 3 diets with varying proportions of vegetables but no egg, supported excellent growth, fair reproduction, but very poor lactation. Growth and fertility were slightly better in the 2nd and 3rd generation than in the 1st, but the percentage of young reared decreased with each successive generation, owing to poor lactation.

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[1] Sherman and Campbell, *J. Biol. Chem.*, 1924, lx, 5.

On the 4th diet, in which egg was used, there was better growth of the young, earlier maturity of the mothers, higher fertility, and better lactation. These findings are summarized in Table II.

TABLE II.
Growth and Reproduction on Diets with 10, 15 and 20% of Vegetables (H, M and L respectively) and with added Egg (E).

	Diet H	Diet L	Diet M	Diet E
Number of young reared	336	318	217	448
Gains per 1000 calories eaten, in gm.	(not calculated)	70	70	76
Av. wt. of young at 28 days, in gm.	43	47	44	45
Gains in wt. 28-56th day, in gm.—Males	69±1.8	80±2.1	75±2.0	87±1.6
Females	55±1.0	55±1.2	55±1.2	65±0.8
Age females at birth of 1st young, days	105±7.8	101±2.3	103±3.5	94±1.0
% of young reared	31	26	21	42
% of females bearing but not rearing young	40	25	32	20
Av. no. young reared per female rearing young	23	16	13	20

These experiments show that a mixed diet already adequate but not containing egg can be improved by the addition of egg more than by an increase in the amount of vegetables, and suggest special supplementing values for the egg, which require further investigation.

4022

Notes on Some Parasites from Chimpanzees.

HAROLD KIRBY, JR. (Introduced by L. L. Woodruff.)
From the Osborn Zoological Laboratory, Yale University.

Through the kindness of Dr. H. C. Bingham and Professor R. M. Yerkes an opportunity was presented to examine for parasites 4 chimpanzees maintained by the Institute of Psychology at Yale University. These animals came from various parts of Africa and have been at the laboratory of the Institute since September 12, 1925, before which time 2 of them were for 3 months in a zoo in Philadelphia.

Since March, 1926, occasional samples of stools from the chimpanzees have been examined, always with the result of finding in large numbers motile forms of a *Balantidium* morphologically indistinguishable from *Bal. coli.* Cysts occurred only very rarely. Although these ciliates have swarmed in the intestines of the animals, at least since examinations first began, and have been passed in great numbers, presumably in every stool, the chimpanzees are healthy and show no visible signs of suffering from their infection. The stools have been more often soft than well formed, and have occasionally been diarrhoeic, but consistency has varied with the animals' diet. In stools kept at room temperature the ciliates have remained alive for several hours, some as long as 32 hours after leaving the host.

Balantidia in anthropoids have been studied by Brooks,[1] Noc,[2] Brumpt,[3] Joyeaux,[4] Christeller,[5] Hegner and Holmes,[6] Ziemann[7] and Cunha and Muniz.[8] As a result of their observations it appears that the ciliates may parasitize orangutans, macaques, capuchins, baboons and chimpanzees; and that the animals are in most cases carriers, not apparently suffering from the presence of the parasites, but may in some cases develop ulcerative dysentery. Dobell and O'Connor state that these animals react to *Balantidium* in much the same way as does man and Hegner[9] says "it has long been known that the organism is pathogenic to primates." Most of the authors have supposed that the species with which they dealt was *Bal. coli.* Hegner and Holmes, however, point out some differences between the *Balantidium* of *Cebus variegatus* and *Bal. coli,* while Cunha and Muniz describe a new species, *Balantidium aragaoi,* from *Cebus caraya* in Brazil. Brumpt parasitized a macaque *(Macacus cynomolgus)* with *Balantidium* from a pig, and Walker[10] infected monkeys with ciliates from both pigs and man. Ziemann twice tried to infect himself by ingesting trophozoites and cysts from chimpanzees, but was unsuccessful.

In these animals, as well as in man, *Balantidium* may sometimes invade the tissues and produce intestinal ulcers. The ulcers have been studied, and in them ciliates demonstrated, by Brumpt, Walker, Noc and Christeller. In many of Walker's experimentally parasitized monkeys the tissues were not invaded. Several fatal cases of balantidiasis in anthropoids have been reported. Thus a family of orang-utans in the New York Zoological Park (Brooks), a macaque at the Pasteur Institute (Noc), and two chimpanzees at the Berlin Zoological Gardens (Christeller, Ziemann) were said to have died of the disease. Diarrhoeic symptoms have frequently been found associated with the presence of *Balantidium,* without evidence that the ciliate was the cause of the condition. Ziemann suggests that infested animals may for a long time be carriers, until *Balantidium* becomes actively pathogenic through lowering of the host's resistance or change in the pH of the intestinal mucus. In the chimpanzees at Yale there are heavy infections apparently unaccompanied by pathological conditions.

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[1] Brooks, H., *Proc. N. Y. Path. Soc.*, n. s., 1903, iii, 28.

[2] Noc, F., *C. R. Soc. Biol.*, 1908, xliv, 878.

[3] Brumpt, E., *C. R. Soc. Biol.*, 1909, lxvii,, 103.

[4] Joyeaux, C., *Bull. Soc. Path. Exot.*, 1913, vi, 612.

[5] Christeller, E., *Virchow's Arch. Path. Anat.*, 1922, ccxxxviii, 396.

[6] Hegner, R. W., and Holmes, F. O., *Am. J. Hyg.*, 1923, iii, 252.

[7] Ziemann, H., *Arch. Schiff. Tropenhyg.*, 1925, xxix, Beih. 1, 434.

[8] Cunha, A. M. Da, and Muniz, J., *C. R. Soc. Biol.*, 1927, xcvii, 823.

[9] Hegner, R. W., *Arch. Path. Lab. Med.*, 1927, iii, 1009.

[10] Walker, E. L., *Phil. J. Sci.*, 1913, viii, sec. B, 333.

Besides *Balantidium* 2 other Protozoa have been found in these chimpanzees. The ciliate, *Troglodytella abrassarti,* was very abundant in fresh feces when the animals were first examined, but a few weeks later it became scarce and during many subsequent examinations was not seen. Recently, 20 months after the first observation, there were still a few present in 2 of the animals. *Troglodytella* perishes much more quickly than does *Balantidium,* especially at room temperature, but a few have remained alive after eight hours in feces kept at 36° C.

In the mouths of 2 chimpanzees a species of *Entamoeba* was found. This closely resembled *E. gingivalis,* and was especially remarkable for its eruptive manner of forming pseudopodia, a characteristic which has seldom, if ever, been recorded for *E. gingivalis.* In the infected mouths there were evidences of pyorrhea, with pockets in which the amoebae were plentiful. Several attempts to cultivate them in the media used for *E. gingivalis* were unsuccessful.

In addition to the Protozoa mentioned above, several intestinal worms have been present. A fragment of a cestode, *Bertiella studeri* (determined by C. W. Stiles) was passed by one ape when the examinations first began, and in the faeces of another eggs of *Trichuris* and *Ascaris* were found.

4023

Antigenic Relationships of Certain Strains of Staphylococcus Aureus as Shown by Agglutinin Absorption.

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From the Departments of Dermatology and of Bacteriology, College of Physicians and Surgeons, Columbia University.

Hemolytic strains of staphylococcus producing a varying amount of yellow pigment are found in pyogenic processes, in septicemias, and also in non-suppurative types of dermatitis and on apparently normal skin. The majority of strains from these varied sources cross-agglutinate in monovalent serums. In an attempt to differentiate them by the method of agglutinin absorption an interesting relationship has been brought out which is in accord with the hypothesis of a multiple antigenic composition for such organisms.

Agglutinating sera were made in rabbits and reciprocal agglutinating absorptions carried out. To obtain constant results it was found necessary to use fresh suspensions of standard strength and even so, specific reactions seemed obtainable only in narrow quantitative limits. If too heavy suspensions were used absorption was obtained which was apparently non-specific. This was due probably to adsorption of the anti-bodies by the bacteria, similar to that which can be accomplished by thick finely divided insoluble suspension of many substances. Tentative grouping could be obtained by testing the varied serums in an optimum dilution, but for clear cut results the following technique was found most satisfactory: A series of dilutions of the serum from 1-10 to 1-12,800 was prepared and each dilution absorbed with a standard dose of fresh cocci. After absorption and centrifugation the supernatant fluid was divided into 2 parts and tested for agglutination; one part against the strain used for absorption and the other against the strain used for production of the serum. With completely analogous strains complete absorption appeared at the same dilution, that is, if all the antibodies for the absorbing strain were removed those for the immunizing strain were also removed. Such a reaction, however, was not always reciprocal and a given strain often absorbed all the agglutinin from the serum from another strain when a suspension from this second strain would not remove all the agglutinin from a serum prepared against the first. In reciprocal tests with three strains A, B and C, it was found that:

A absorbed all the agglutinins from serums prepared against A and B.

C absorbed all the agglutinins from serums prepared against B and C.

B failed to absorb all the agglutinins from either A or C.

This could be explained on the hypothesis that strain A contained agglutinogenic substances A and B; that strain C contained agglutinogenic substances B and C; but that strain B contained only agglutinogenic substance B. It is realized that such a statement is probably a crudely symbolic method of indicating the difference in physico-chemical composition of these different races.

Many other strains have been encountered which on simple absorption tests seem to fall into each of these 3 groups. Agglutinating sera had been prepared against 1 or 2 representatives of each group and on reciprocal absorption tests the strains appeared completely homologous with those on which tests are here recorded. These strains therefore probably represent groups and not individual variants. So far, no members of groups B or C have been found in frankly pyogenic processes, but the number tested is insufficient to make this finding more than suggestive. Similar relationships have been found by Krumwiede and others in the Gram-negative bacilli but these findings are here reported because they present an unusually clear-cut instance of such reciprocal agglutinating relationships.

4024

Immunologic Reactions with Tobacco Mosaic Virus.

HELEN A. PURDY. (Introduced by F. P. Gay.)

From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, and the Boyce-Thompson Institute for Plant Research, Inc., Yonkers, N. Y.

A study of the immunologic reactions with a tobacco mosaic virus to an antiserum from rabbits has been undertaken with the hope of throwing more light upon the nature of the virus.

Antisera were produced separately by intravenous injections of rabbits with sap extracted from healthy Turkish tobacco plants, and sap from plants affected with tobacco mosaic virus. In several series of alexin-fixation experiments, each antiserum was titrated with its homologous and heterologous antigen. The higher titer for a given antiserum was obtained with the homologous antigen. These results are in agreement with Dvorak,[1] who compared the precipitin reactions of an antiserum to healthy potato sap with those of an antiserum to sap extracted from mosaic diseased potato plants.

Precipitin-absorption tests further demonstrated the presence of a highly specific precipitin in the antiserum to virus-sap.

The preventive action of specific antiserum upon virus-sap *in vitro* was demonstrated by the inability of antiserum-virus mixtures, that had stood at room temperature for several hours, to induce mosaic disease when inoculated into healthy tobacco plants. Under the same set of conditions, normal rabbit serum and antiserum to healthy tobacco sap did not exhibit the preventive action upon virus that the specific antiserum showed. Fresh antiserum to virus-sap exerted a stronger inhibiting action than the same antiserum, inactivated by a temperature of 56° C. for one-half hour. Heated antiserum could be reactivated by the addition of fresh rabbit or guinea pig serum, so that the reactivated antiserum produced the same preventive action upon virus as the active antiserum.

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[1] Dvorak, M., *J. Infect. Dis.*, 1927, xli, 215.

Pacific Coast Branch.

Stanford University, April 28, 1928.

4025

Further Studies on Penetration of Methylene Blue.*

MATILDA MOLDENHAUER BROOKS.

From the Zoological Laboratory, University of California.

In a former paper[1] it was shown that when the marine alga, *Valonia,* was placed in solutions of methylene blue dissolved in sea water at pH's 5.8 and 9.0, the dye penetrating into the sap was methylene blue itself and not some lower homolog, such as trimethyl thionin, formed by the oxidation of methylene blue in the outside solution. The identification of the dye was made by means of the spectrophotometer.

In order to test this conclusion still further, another set of experiments was made using the fresh water alga, *Nitella.* The method was similar to that used in the experiments with *Valonia.* The same sample of dye was also used. All necessary precautions were taken during extraction to keep the sap from becoming contaminated by dye from the rest of the cell. The plants were also tested for injury by transferring samples from the experimental solutions to tap water. These were found to be alive (green and turgid) 2 months after experimentation, when they were discarded.

In the experiments herewith reported selected *Nitella* cells of the same size were placed in solutions of methylene blue for 2 hours at 25° C., after which the sap was extracted, placed in a hollow ground slide with a flat-bottomed depression, covered by a cover slip, and spectrophotometric measurements made.

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* Part of the expenses of this investigation were defrayed by grants from the Bache Fund of the National Academy of Sciences and from the Research Fund of the Sigma Xi.

[1] Brooks, M. M., *Univ. of Calif. Pub. in Zool.,* 1927, xxxi, 79; *Proc. Nat. Acad. Sciences,* 1927, xiii, 821.

It was found that the dye penetrating into the sap had a maximum absorption at 660 mμ and a secondary maximum at 610 mμ. These are the absorption maxima for methylene blue. It is therefore concluded that methylene blue penetrates *Nitella* sap as such. This corroborates the previous results obtained by the writer with *Valonia*.

4026

Persistence of Denatured Horse Proteins in the Canine Circulaton.[*]

J. L. AZEVEDO AND W. H. MANWARING.
From the Laboratory of Bacteriology and Experimental Pathology, Stanford University.

In previous papers[1, 2] it was shown that horse proteins injected intravenously into normal dogs are retained quantitatively in the canine circulation for at least 6 days. During this retention, however, the proteins undergo marked denaturization. By the end of 4 days they will call forth no recognizable anaphylactic reaction even on massive transfusion into hypersensitive dogs. By the end of 6

TABLE I. *High Dilution Tests.*
Dogs injected intravenously on different days with 2 cc. horse serum per kg. of body weight. Blood samples withdrawn on the same day. Sera from these samples tested in parallel dilutions with rabbit precipitin. (For details of technic see previous papers.) +++, ++, +, (+), visible precipitate; ttt, tt, t, (t), (?), turbidity or opalescence; 0, no demonstrable reaction.

Dilution	6-day Sample	30-day Sample
1: 32	++(+)	tt
1: 64	++(+)	(+)
1: 128	++	+
1: 256	++	+
1: 512	+(+)	+
1: 1,024	+	(+)
1: 2,048	+	(+)
1: 4,096	(+)	(+)
1: 8,192	tt(t)	(+)
1: 16,384	t(t)	tt
1: 32,768	t	t
1: 65,536	(t)	(t)
1:131,072	0	0

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[*] Work aided by a grant from the Committee on Scientific Research of the American Medical Association.

[1] Manwaring, W. H., Marino, H. D., McCleave, T. C., and Boone, T. H., *J. Immunol.*, 1927, xiii, 357.

[2] Manwaring, W. H., Marino, H. D., and Azevedo, J. L., *J. Immunol.*, 1928, xiv, in press.

days they cease to act as sensitizing agents for normal dogs. We have endeavored to follow subsequent changes in these proteins.

Titration by means of a specific horse-protein-precipitating rabbit serum indicate that the proteins are further altered, denaturized or inhibited. By the end of 30 days they give little or no demonstrable reaction with rabbit precipitin, if tested in low dilutions.

That this negative reaction is not due to an absence of horse proteins from the 30-day blood sample is indicated by continuing the titration to higher dilutions (Table I). In dilutions above 1:1000, the 6-day and 30-day samples give practically identical precipitates and turbidities.

The horse proteins thus further altered are apparently retained quantitatively for at least 4 months (latest titration thus far made). Within the limits of the experimental error the 4-month and 30-day blood samples give identical precipitates in all dilutions.

4027

A Rubber-Opposed Haemodromograph Used to Measure Reflux in Aortic Insufficiency.

W. DOCK AND J. J. O'HARA.

From the Department of Medicine, Stanford University School of Medicine.

The measurement of blood flow throughout the cardiac cycle has been accomplished by use of instruments of rather low efficiency due to their heavy construction and the use of more sensitive instruments has been restricted by the difficulties attending their use. We have attempted to construct a simple instrument for use in studying reflux. The lever of the aortic haemodromograph is of fine aluminum wire, the fulcrum a water-tight strip of rubber dam fixed between 2 metal plates. An oval aluminum vane, 6x8 mm., fixed at one end of the lever projects into the aorta at right angles to the direction of flow; the connection is made air-tight by an oval shoe and threaded washer (Fig. 1). The length from vane to fulcrum is 2 cm., from fulcrum to the strand of rubber used to damp the lever is 5 cm., and a fine brass wire projects 3 cm. above this, its shadow registering on the moving film. The haemodromograph for the carotid is incorporated in the side of a brass tube, 4.5 mm. in diameter, which is introduced between cut ends of the vessel. The lever is damped 5 mm. from its fulcrum, the vane extends 4 mm.

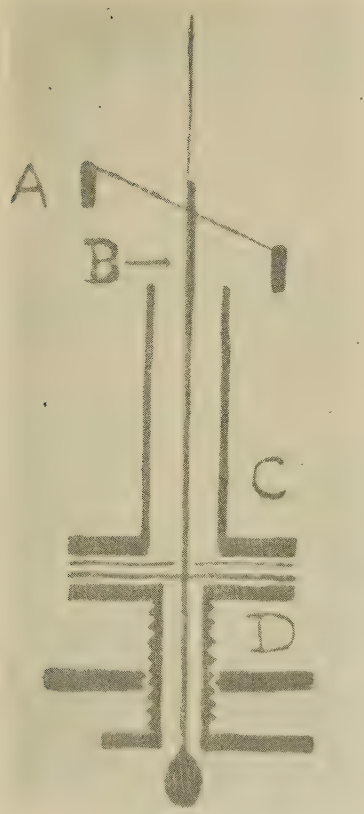

FIG. 1.

Schematic diagram of aortic haemodromograph. A. Rubber strand at right angles to direction of flow. B. Lever with vane in aorta and fine wire projecting into beam of light. C. Upper half of canula. D. Lower half with shoe at opening and threaded washer, between which the aorta is held. Between C and D is a piece of rubber dam and a perforated metal plate forming the fulcrum for B.

into the blood stream and the fine wire extends 3.5 cm. from the fulcrum. The calibration of the apparatus, by measuring deflection produced by known rates of constant flow, is theoretically simple and accurate. In practice with our rather crude instruments we have found such calibration misleading, as the deflection is not constant from day to day, and small wisps of fibrin deposited on the vane produce a marked deviation. At present it can be used only for studying the shape of the curve of rate of flow. The instruments used have a vibration frequency of 50-80 per second; decrement per beat is from 20-30%. The sensitivity and decrement can be varied by varying the tension of the elastic strand. The relationship between deflection of the lever and rate of flow is not linear. With increasing velocity the sensibility of the instrument for a given increment of speed is increased.

In Fig. 2, it will be noted that there is no movement of blood in the aorta in diastole, in fact there seems to be a slight back-flow at the end of systole. The lever is vibrated by the closure of the semilunar valves. In the carotid there is a slight but appreciable flow of blood in diastole, but the curve is much like that in the aorta. Destruction of one or more aortic leaflets, in acute experiments, produces a definite back-flow of blood in the aorta (Fig. 3). With two leaflets destroyed the regurgitation equals or surpasses 50% of the total blood ejected in systole. The proportion of regurgi-

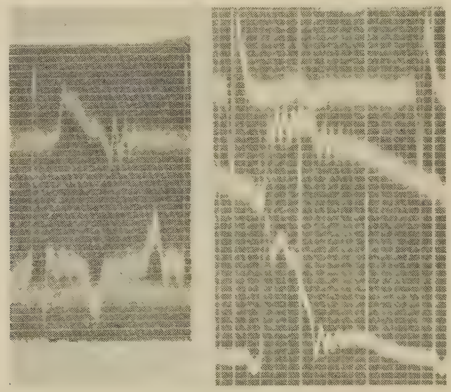

FIG. 2.

A. Upper curve, rate of flow in aorta, lower E. K. G. The peak of flow equals about 800 mm. per second. B. Upper curve E. K. G., middle blood pressure, lower rate of flow in carotid. Time 1/5 and 1/25 seconds.

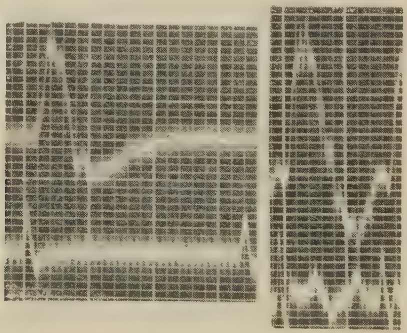

FIG. 3.

Lower curves E. K. G., upper curves aortic blood flow after destruction of 2 aortic cusps. From different animals. The peak of flow in both is about 1200 mm. per second; the damping is greater in the first curve and the circulation more depressed by anesthesia and operation.

tated blood varies with the state of the circulation, being least when the animal is in shock. The destruction of 2 leaflets, in such acute experiments, is fatal in from 30 to 60 minutes. These curves were obtained from dogs anesthetized with chloretone and heparinized after exposure of the great vessels.

4028

Effects of Daily Pituitary Heterotransplants on an Hypophysectomized Puppy.

FREDERICK LEET REICHERT.

From the Halsted Laboratory of Experimental Surgery, Stanford University Medical School.

Recent results obtained by Smith and Engle[1] by daily hypophyseal homeo and heterotransplants in mice and rats led to an investigation of the effect of daily injections of fresh rabbit pituitary gland on an hypophysectomized puppy. Aschner[2] published striking photographs of hypophysectomized puppies of various ages which had remained infantile in appearance and size until sacrificed.

Evans and Long[3] found that fresh anterior lobe fluid given intraperitoneally to immature rats produced gigantism. Later, this extract was found to stimulate hypophysectomized rats to normal growth but failed to affect their sex glands. Smith,[4] using fresh pituitary glands of rats, was able completely or in large measure to cure the disabilities arising from hypophysectomy in immature rats. Smith and Engle[5] were able to induce precocious sexual maturity both in immature and in hypophysectomized rats, in 2 animals within 36 hours, by these fresh daily transplants. By means of the intracranial approach of Dandy and Reichert[6] a total hypophysectomy was performed on a 6 weeks old female puppy and the base of the brain in the infundibular region cauterized by heat. This animal was constantly kept with her control litter mate, also female. After months of observation, she remained infantile, failed to grow, retained the puppy face and hair, failed to erupt permanent teeth and exhibited infantile genitalia, indicative that a total hypophysectomy had been performed.

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[1] Smith, P. E., and Engle, E. T., *Am. J. Anat.*, 1927, xl, 159.

[2] Aschner, B., *Arch. f. d. Ges. Physiol.*, 1912, cxlvi, 1.

[3] Evans, H. M., and Long, J. A., *Anat. Rec.*, 1921, xxi, 62.

[4] Smith, P. E., *J. Am. Med. Assn.*, 1927, lxxxviii, 158.

[5] Smith, P. E., and Engle, E. T., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 561.

[6] Dandy, W. E., and Reichert, F. L., *Bull. Johns Hopkins Hosp.*, 1925, xxxvii, 1.

Six months after hypophysectomy when 7½ months old, weighing 4.2 kilos and when the control had attained adult size and weighed 9.3 kilos, fresh whole rabbit's hypophysis was given subcutaneously every day by the technic described by Smith and Engle.[1] Within 48 hours the vulva were definitely swollen and within 3 days became so greatly distended as to protrude 3 cm. from the body. This marked swelling of the external genitalia has now persisted for over 2 months while the control whose external genitalia have remained of normal size has never been in heat. A vaginal secretion appeared after about 2 weeks but she did not attract a male dog until the 18th day when coitus was attempted.

The weight of the experimental puppy remained stationary for the first 2 months, then began to increase, although adipose tissue in the groin definitely disappeared during the first 2 weeks of giving the transplants.

During this 2 months' period of treatment, measurements of the long bones outlined by the X-ray have shown no growth in the control, and an increase in length of the tibia and femur of only 1 cm. each in the operative animal.

The permanent teeth appeared during the 3rd to 5th month in the control, while even at the age of 7 months the hypophysectomized puppy still had all of the first denture. Within a month after the beginning of the injection therapy these teeth dropped out and were replaced by the permanent set.

The experimental animal showed a marked change in behavior, with increased activity, attentiveness and playfulness appearing early in the first week of treatment.

In the 3rd week the soft, downy texture of the coat began to change to a coarser and longer coat of hair.

This heterotransplant treatment has readily produced the characteristic stimulation of the genital system, and has led to maturity of other systems, while skeletal growth was so far but moderately affected. The latter might be attributed to the fact that an interval of 6 months existed between the time of hypophysectomy and the initiation of the replacement therapy during which time the control had attained maturity.

Further experiments are in progress to determine the effect of transplants on puppies soon after hypophysectomy.

4029

Specific Rotation and Phosphate Content of Cold-Water-Soluble Fractions of Ground Corn and Wheat Starches.

JOHN FIELD, 2D. (Introduced by C. L. Alsberg.)

From the Food Research Institute and the Department of Chemistry, Stanford University.

Starch, as it occurs in nature, is not soluble in cold water, and hence one cannot determine the specific rotation of the untreated substance. The several soluble starches, which have formed the basis of numerous optical studies, represent natural starches altered either by heating or treatment with reagents, or by treatment with enzymes, or by some combination of the above procedures.

The reported values of the specific rotation of the various starches range from 216.0° to 182.0°. This wide range of results indicates that the determinations were made on starches hydrolyzed to a varying extent, or on untreated solutions so opalescent as to prevent accurate evaluation of the specific rotation.

It was shown by Alsberg, Griffing, and Field[1] that clear solutions could be prepared from purified starch by dry-grinding in a pebble-mill, dispersion in cold water, and centrifugation. This technique is particularly effective with wheat and corn starches. These solutions are water-clear, dialyze through collodion, and give an intense blue with iodine. Such solutions are always very dilute.[2, 3]

The specific rotation of these solutions has been determined. The source of light employed was an electric lamp of the type described in Circular 44, U. S. Bureau of Standards, page 34. Determinations of the concentration of the starch solutions were made by the method of Munson and Walker.

The average of a number of determinations gave 196.0° for the specific rotation of the cold-water-soluble portion of ground corn starch, and 194.7° for wheat starch, with the light-source specified. This difference is not significant in view of the fact that it lies in the range of analytical error in determining the concentrations of such dilute solutions. These determinations afford no evidence that the cold-water-soluble fractions of ground corn and wheat starches differ in character.

Furthermore it has been found that the cold-water-soluble fractions of ground corn and wheat starches contain no phosphate, which confirms some earlier observations by Samec.[4] The recently published method of Embden was used in testing for phosphate.[5]

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[1] Alsberg, C. L., Griffing, E. P., Field, J., *J. Am. Chem. Soc.*, 1926, xlviii, 1299.

[2] Alsberg, C. L., *J. Ind. and Eng. Chem.*, 1926, xviii, 190.

[3] Field, J., and Becking, L. G. M. B., *J. Gen. Physiol.*, 1926, ix, 445.

4030

Induced Encystment and Excystment of a Marine Euplotes, Sp. Nov.

LAURA GARNJOBST. (Introduced by C. V. Taylor.)
From the Biological Laboratories of Stanford University.

A new species of Euplotes, a marine hypotrichous ciliate, found in San Francisco Bay can be readily induced to encyst and excyst at will by the following method.

By means of a micro-mouth pipette, from 25 to 35 organisms picked at random from a thriving culture are placed on a slide or coverslip within a drop of the culture medium. The slide (or coverslip) is then placed in a moist chamber to allow gradual evaporation to take place. The rate of evaporation under these conditions at about 22° C. gives the best results. Within 1 to 10 hours, or even less time, encystment occurs. This process includes a gradual decrease in motility of the organisms until they become fixed to the slide, whereupon they gradually assume a rounded form. External organelles at first can be recognized within the cyst wall but later disappear from view. Excystment takes place when a small drop of fresh culture medium, tap, or distilled water is added to the drop containing the cysts. The time required for complete excystment depends upon the age of the cyst and varies from a few minutes to 1 or 2 hours. A contractile vacuole reappears and functions slowly soon after the addition of water. The cyst wall breaks, usually just above the contractile vacuole. The contents stream to the outside, external organelles make their appearance, and soon the organism again becomes free-swimming.

The above method has proved to be an excellent means for studying the structural changes which occur during cystment. The coverslip is inverted upon a depression slide, the edges sealed with vaseline, and now the slide may be placed under a microscope. There the processes of encystment and excystment can be studied with any magnification, including oil immersion. This coverslip method provides also a means of making permanent slides without danger of cysts becoming detached at any time during the staining process, and prevents injury to the cysts due to transference. Obtaining accurate data regarding age of individual cysts is also made possible by this method.

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[4] Samec, M., *Koll. Chem. Beihefte,* 1914, vi, 23.

[5] Embden, G., *Z. fur physiol. Chem.,* 1921, cxiii, 138.

A study of (1) living material and (2) stained preparations of cysts in various stages has brought out the following facts: During some period of encystment external organelles disappear. With excystment a redifferentiation of these structures occurs. The nuclear apparatus undergoes a reorganization process during encystment which is similar to that taking place in binary fission and conjugation in this species.

4031

Regeneration of Peridental Membrane in the Cat.*

T. D. BECKWITH AND ADRIENNE WILLIAMS.

From the Department of Bacteriology, University of California.

Recently we[1] have shown that regeneration of the peridental membrane takes place in the guinea pig and rabbit following artificial destruction of this tissue. The sequence of events leading up to this repair was outlined. Injury had been induced by passing a dental bur through the alveolar process across the peridental membrane and into the upper incisor tooth on its lingual surface. Thus, the peridental fibres were severed at this point. The rodent was chosen for that work since it has continuously growing teeth with the result that uninjured tooth surface will move forward to a position opposite to the site of injury to the alveolar bone. Thus repair may be followed more easily by microscopic technique.

A similar series of experiments then was performed upon cats. This was done in order to learn whether a like sequence of repair may be demonstrated in an experimental animal still higher in scale and in which the tooth is stationary. The animals were 4 or 5 months old and had received the usual animal house diet. The operation was performed by Dr. F. V. Simonton of the College of Dentistry of this university and his cooperation is thus acknowledged.

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* Aided by grant from the Carnegie Corporation, the American Dental Association and the Associated Radiograph Laboratories of San Francisco.

[1] Beckwith, T. D., Williams, Adrienne, and Fleming, W. C., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 562.

Following the operation, the animals were killed with chloroform as follows: 10 days, and 7, 8, 13, 17, and 20 weeks. The experimental tissues were then dissected out and sections cut according to the method outlined by one of us (A. W.).[2]

Examination of the sections made shows a picture closely similar to that found in rodent tissue. In certain of our animals killed at the close of the 7 and 8 week periods, it was evident that infection had developed. This had resulted in destruction of tissue adjacent to the site of the cut and both hard and soft tissues had been involved.

Destruction in turn had been followed by repair. New cementum had been laid down upon the external surface of the eroded dentine. As this cementum was formed fibroblastic migration took place upon the cementum surface. Fibroblasts in turn developed into true fibrous structure. These fibres extended across the field to newly forming alveolar bone and as the bone repaired these fibres had become incorporated within it. Repair of the alveolar bone progressed in the usual manner by osteoblastic activity. It is of interest to note that the alveolar tip extended into the area of previous destruction rapidly but, on the other hand, growth of the bone

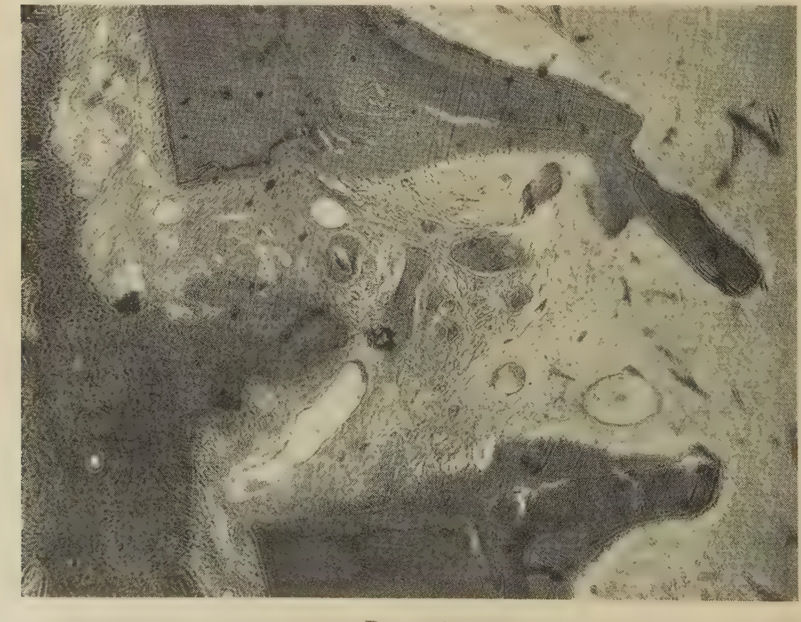

PLATE 1.
Repair of peridental membrane in cat. Note extension of alveolar bone into the cut area. Cat. 8 weeks.

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[2] Williams, Adrienne, *Dental Cosmos,* July, 1927.

towards the tooth was a slower process. This resulted in a longer exposed span for the newly formed peridental fibres. It was evident, however, that the new fibres were becoming incorporated within this bone.

Plate No. 1 indicates repair fundamentally like that noted just previously. In this animal a thick formation of cementum had been laid down upon the cut wall of dentine. In this instance, however, the alveolar wall has extended into the cut and thus an hypertrophy of the alveolar bone has been produced. Peridental fibres connect the newly formed cementum with this projection of bone.

Replacement of true peridental membrane and not merely scar formation thus has been shown in the cat following initial destruction by artificial means.

4032

Hypertrophy of Mammary Gland in Adult Male Rats with Experimental Ovario-testes.*

EARL T. ENGLE.

From the Department of Anatomy, Stanford University.

In a previous report[1] it was stated that typical ovario-testis could be produced in adult male rats by grafting the ovaries of immature females into the testis, subsequently giving daily transplants of the anterior lobe, using Smith's technique.[2]

Eight to 12 days of treatment is sufficient to produce numerous ovarian follicles of mature condition in the ovarian graft, but no changes have been observed in the host animal. If the daily transplants are continued for 18 days, the ovarian mass engrafted in the testis is greatly increased. The large follicles are more numerous, and in many cases, the thecal cells have hypertrophied, forming a layer of lutein cells around the periphery of the follicle.

As shown in Fig. 2, the response in the tissue of the mammary gland of the treated male rat bearing an ovarian graft is markedly different from that of its litter-mate (Fig. 1), which had received the same number of daily transplants of anterior lobe, but which did not bear an ovarian graft.

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* Aided by a grant from the National Research Council Committee for Research in Problems of Sex.

[1] Engle, E. T., PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 83.

[2] Smith, P. E., *Am. J. Physiol.*, 1927, lxxx, 114.

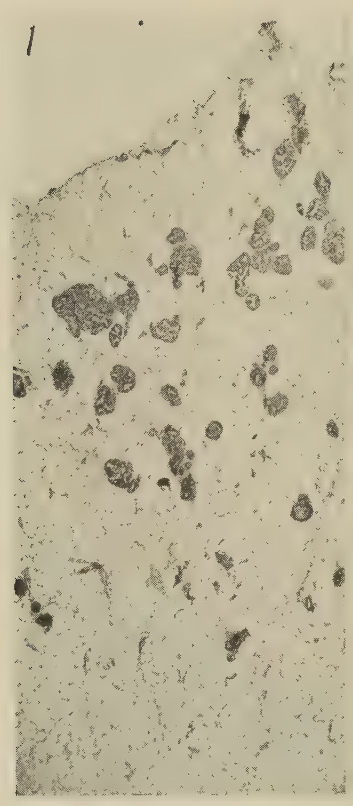

FIG. 1.
Mammary gland of male rat, 184 days old. Daily pituitary transplants, 18 days. No ovarian graft. x25.

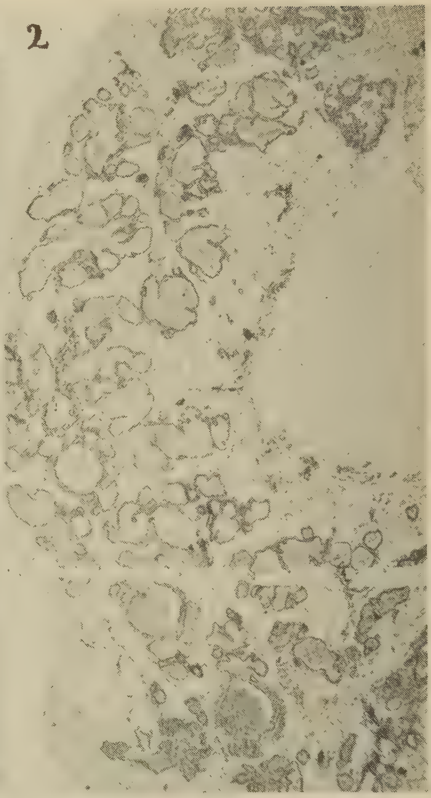

FIG. 2.
Mammary gland of male rat, 184 days old. Daily pituitary transplants, 18 days. Ovarian graft in each testis, each graft having many follicles. x25.

These rats possess normal seminiferous tubules, and have mated with normal females during the experimental period. All animals bearing grafts have sired 1 litter, many 2 litters, and a few 3 litters, during the period of the experiment.

4033

Skin Transplantation in Ducks and Pigeons.

C. H. DANFORTH.

From the Department of Anatomy, Stanford University.

In view of the finding that skin can readily be transplanted in young chicks[1] it seemed desirable to see if a similar procedure would be equally feasible in other commonly used laboratory birds. To this end a few experiments were made with newly hatched ducks and pigeons.

Skin from 2 young mallards supplied grafts for 6 white Pekin ducklings. Of these, 1 graft to the back and 1 to the breast were entirely successful, 2 to the top of the head and 1 to the back failed, and 1 duckling was lost before the fate of its graft could be determined. Mallard skin grown on a Pekin host has produced juvenile mallard plumage.

Through the kindness of Professor C. S. Stoltenberg 20 pigeons were available for the second experiment. Four autoplastic grafts were all successful. Of the exchange grafts between full brothers and sisters, 3 became fully established and 7 others lived for 9, 11, 12, 15, 26, 30+ and 30+ days. When donor and host were not closely related, the grafts lived for 7, 7, 9, 12, 17 and 25+ days.

Because of the rapid growth of the young and the ease with which the condition of the graft may be determined from day to day without the necessity of sacrificing the host, the pigeon seems especially favorable for the study of graded compatibilities of tissues. The data thus far available indicate a sharper differentiation of individuality factors in the pigeon than in the fowl. There is evidence that the grafts which are doomed to fail may nevertheless have varying life expectancies depending upon the part of the body to which they are transplanted.

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[1] Danforth, C. H., and Foster, Frances, PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 75.

4034

Effects of High Frequency Sound Waves on Protoplasm.

F. O. SCHMITT,* A. R. OLSON AND C. H. JOHNSON.
(Introduced by T. D. Beckwith.)

From the Department of Chemistry of the University of California.

The observations described in the following preliminary report were made by subjecting animal cells to the action of sound waves produced by a rapidly vibrating quartz crystal. The oscillating current was generated by a 250 watt tube connected in a modified Hartley circuit. The crystal lay between a heavy lower electrode and a light upper electrode of copper gauze in a crystallizing dish containing the dielectric liquid, usually xylene. The crystal vibrated at the rate of approximately 750,000 times per second.

Effects in Vitro. In this type of experiment the animals were placed in a test tube or similar vessel containing water and dipped into the dielectric over the vibrating crystal. Free-swimming *Triturus* larvae are rapidly killed, the gill filaments being torn away and areas of denuded surfaces exposed. Confirming the findings of Wood and Loomis,[1] the larger protozoa are killed by this method, while the smaller protozoa, being able to swim between the nodes of the stationary wave system set up in the water, escape unharmed. *Spirostema,* being relatively large, are quickly killed, *Paramecia* less rapidly, while the smaller protozoa are unaffected after many minutes. By means of a simple arrangement it was possible to observe the cells through the microscope while subjecting them to the sound waves. A drop containing the protozoa was placed on a microscopic slide and a cover glass laid over it. The slide was then laid across the oscillator dish. The sound waves were transmitted in this manner to the cells through the medium of the oscillator dish and the slide. Neglecting morphological details for the present, cell destruction is due to an actual disruption of the whole or of a part of the cell, to a gelation or coagulation of the protoplasm, and to a combination of the two effects. Short exposures of protozoa result in a sort of preliminary paralysis which rapidly goes over to death if the exposure is continued. In an effort to assign some cause for the death of protozoa in the absence of visible disruption of the cell, filtered solutions of crystallized egg albumin were subjected to the vibration. In a very few minutes the solution became turbid and the albumin precipitated out in fine shreds. That great pressures may precipitate proteins in the absence of any great heating was noted by Bridgman.[2] We venture to suggest, on the basis of our experiments, that a similar denaturation of the proteins in protoplasm may be an important factor in the lethal effect of sound waves. This does not exclude a thermodynamic pressure-temperature coefficient.

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* Fellow in Medicine of the National Research Council.

[1] Wood, R. W., and Loomis, A., *Phil. Mag.*, 1927, iv, 417.

Supersonic Micromanipulation. The searing effects of sound vibrations conducted along capillary tubes have been demonstrated by Wood and Loomis. We have attempted to devise a method of micromanipulation in which the end of the capillary tubing coming from the vibrating collector is pulled to a fine micropoint and, held in a micromanipulator, is directed at single cells under the microscope. Owing to the limited power at our disposal, it became necessary to find conditions under which maximum energy could be transferred to the collector. It was discovered that when a flat strip of glass, drawn to a thread at one end, was used, maximum heating of the thread resulted when the strip made a certain critical angle with the plane of the crystal. There appears to be a definite relationship between this angle and the velocities of sound in the dielectric liquid and the collector. This relationship offers an extremely simple method of studying the velocity of sound in various media at these frequencies; this phase of the subject is now under investigation in this laboratory.

Planarian worms touched even though lightly by the tip of such a vibrating needle are instantly burned, the effect manifesting itself in the form of a cut, which is produced at the point of contact. The edges of the cut appear to be smooth and the pigment recedes from the immediate neighborhood of the incision. If the shank of the needle be placed across the worm, immediate cutting takes place and the body of the worm is severed.

A difficulty is encountered when the attempt is made to insert the vibrating needle into single cells. Instantly when the needle is brought up into the hanging drop, although no motion of the needle itself is apparent, cells and suspended debris are rapidly rotated about the needle and are finally flung away from the vicinity. Under controlled conditions this effect is being utilized to cause general vibration in cells; conjugants, for example are rapidly separated with no apparent ill effects.

By modification of the attachment of the needle it became possible to insert the point into amebae and other free cells and to observe the effects on protoplasm. Full account of these observations is reserved for future communication but preliminary experiments indicate that insertion of the needle produces alteration of the viscosity of the protoplasm. Provided that the treatment is not too intense or too prolonged, effects appear to be reversible. More intense treatment results in local injury, and in extreme cases complete disorganization or even disruption of the cell.

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[2] Bridgman, P. W., *J. Biol. Chem.*, 1914, xix, 511.

Some time after this communication was submitted for publication, on April 28, our attention was called to a paper by Harvey and Loomis, published in *Nature* for April 21, entitled "High Frequency Sound Waves of Small Intensity and their Biological Effects." Although some of the observations therein described parallel closely results of some of our earlier, as yet unpublished experiments, we should like to point out that our method of affecting single cells differs distinctly from theirs; in our expriments, the supersonic effects are, for the most part, applied in definitely localized regions of cells by means of glass collectors pulled to micro-points. Data already in hand indicate that such localized disturbances may be followed by deep-set ontogenetic alterations.

4035

Effect of Liver Extract on Erythrocytes and Reticulocytes in Normal Individuals.

CHARLES H. WATKINS, RICHARD JOHNSON AND HILDING BERGLUND.

From the Medical Service of the University Hospital, Minneapolis.

The effect of liver extract in pernicious anemia may be analyzed from the point of view of the immediate reaction, as well as of the sustained reaction. The immediate reaction includes among other things 3 distinct morphological features: (1) the normoblastic response, (2) the reticulocyte response, and (3) the liberation of the mature erythrocytes.[1] It is a well established fact that the bone marrow in pernicious anemia is hyperplastic and contains large amounts of erythrocytes in various stages of development. In other words, there appears to be a difficulty in the maturation of the erythrocytes necessary for their discharge from the marrow into the circulation. This faulty maturation is in character probably morphological rather than chemical, for the cells in the marrow are completely filled with hemoglobin and products of hemoglobin have been deposited in the various tissues. Thus the hemosiderosis, in the classical interpretation of pernicious anemia looked upon as a proof of the hemolytic character of the disease, might indicate a destruction of erythrocytes in the foci where they were formed before they were ever delivered into the circulating blood. When liver therapy is instituted, the bone marrow presumably is stimulated to mature and release the cells. A slight retardation in the maturation as compared with the discharge thus explains the 3 features of the immediate reaction. Experiments on the effect of liver extract upon normal individuals are here presented as a confirmation of the view of the mode of action of the liver extract to accelerate the discharge of cells into the circulation.

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[1] Watkins, Charles H., and Berglund, Hilding, PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 206.

Six normal individuals (doctors and assistants in our laboratory) whose erythrocyte counts were between 4,500,000 and 5,800,000 were given 3 vials of liver extract (343 Lilly), or the equivalent of 300 gm. of raw liver, daily for a period of 10 days.

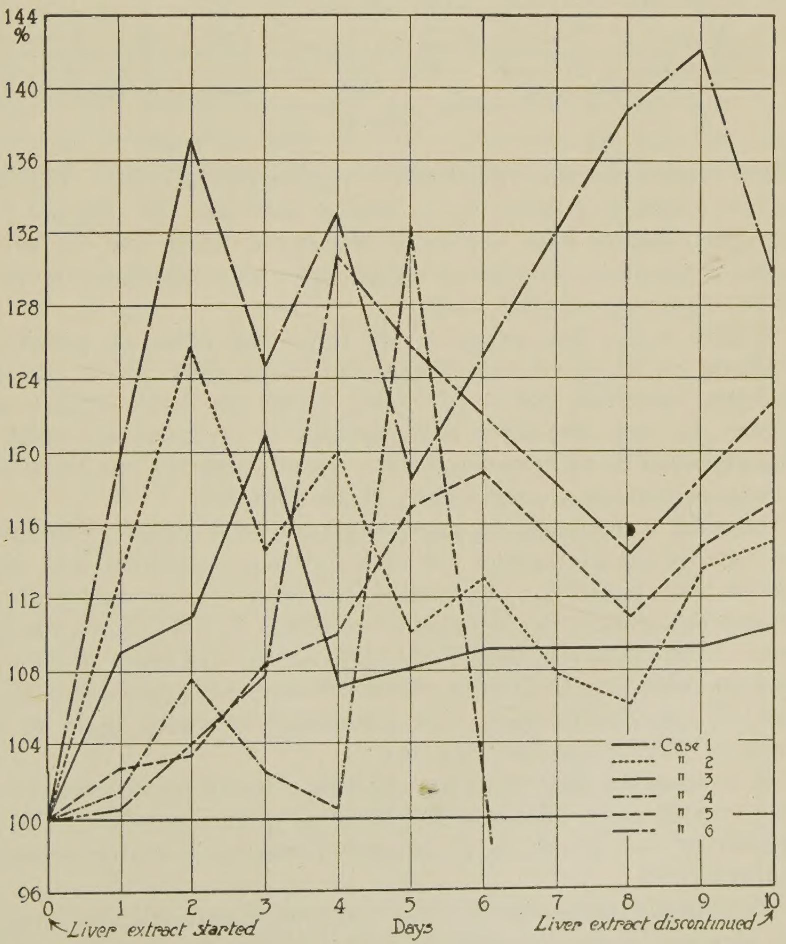

CHART 1.

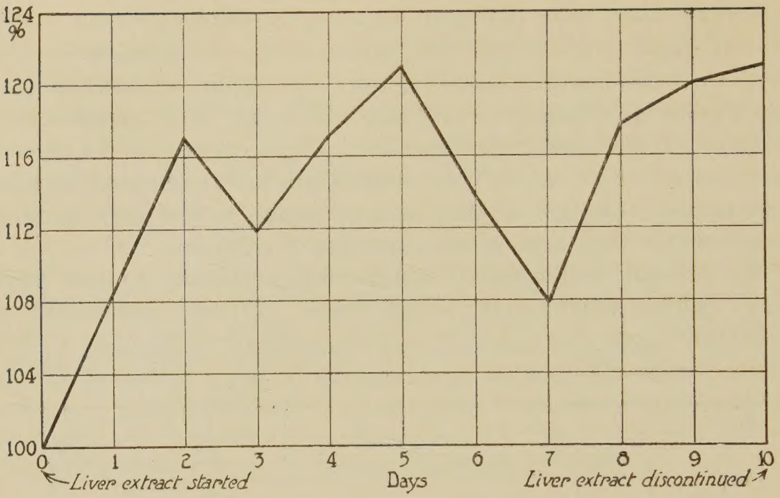

CHART 2.

Daily erythrocyte and reticulocyte counts were obtained. That the results might be reduced to a common base line, the original erythrocyte count of each individual was called 100% and the subsequent increase or decrease in erythrocytes was calculated in terms of the % of the original value.

Figure 1 is a field graph of the individual cases, in which the ordinate represents the percentage increment over 100% and the abscissa represents the days during which the liver extract was given. In each case there is an increase in erythrocytes, which is characterized by an irregular curve. In all cases but one there was a definite increase in erythrocytes at the 10th day.

In order to facilitate the interpretation of the results a numerical average of the percentage for each day was calculated and these values were plotted as represented in Fig. 2. From this graph it is seen that there is an increase from 100% to 117.1% on the 2nd day. Then there is a drop to 111.7% on the 3rd day. Following this the value rises to 120.9% on the 5th day, and again decreases to 107.8% on the 7th day. The percentage increases again to the value of 120.7% on the 10th day.

It is apparent that there is a definite increase in the erythrocyte count in all cases although the curve is quite irregular. The irregularity may be due to an attempt of the body to overcome the polycythemia.

In clear contradistinction to the occurrences in pernicious anemia, no increase in the reticulocytes took place in the normal individuals, the percentage remaining between 0.0% and 2.0%. There was no change in the leukocyte counts. The hemoglobin followed the red count, but probably not in full proportion to the increase in red cells. In all cases the individuals developed some symptoms of polycythemia vera; thus varying degrees of headaches, epistaxis, acrocyanosis and abdominal distress were encountered. We call particular attention to the acrocyanosis.

We call attention to the immediate response in the normal individuals as compared with the delayed response in the discharge of mature red cells in pernicious anemia. If in the pernicious anemia we base our comparison on the occurrence of increased number of normoblasts, the first signs of response to the liver come as early in one group as in the other. Thus the liver extract in normal individuals, as in pernicious anemia brings about an immediate release of red blood cells into the circulating blood. In the normal individual the maturation of the cells is complete and the release rapid, in the pernicious anemia the maturation is retarded, discharge of incompletely matured cells occurs and precedes the discharge of fully matured ones; the process as a whole is prolonged.